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Diatom communities in freshwater springs with contrasting geology in the Apuseni Mountains

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Abstract. Freshwater springs are unique and fragile aquatic ecosystems characterized by a mosaic of microhabitats, hydrochemical properties influenced by geological substrate, and specialized biological communities. Springs from the Romanian Carpathian Mountains are poorly explored and the role of geological substrate in structuring diatom assemblages remains unclear. This study aims to characterize diatom communities and their dynamics in ten springs developed on contrasting geological substrates. Epibryon and epilithon samples were collected from at least three points of available microhabitats within the spring area. Standard methods were applied to obtain cleaned diatom material and the resulting assemblages were analyzed using multivariate ordination techniques, alongside ecological indicator values and functional guild structure. A total of 126 diatom taxa were identified and three of the investigated springs are reported here for the first time with respect to their diatom flora. Community composition differed significantly between the two contrasting groups of springs. Crystalline–metamorphic springs were associated with assemblages dominated by taxa typical of acidic and weakly mineralized waters, whereas carbonate springs supported communities characteristic of more alkaline conditions. Low-profile guilds dominated carbonate springs, whereas one crystalline–metamorphic spring showed a distinct high-profile-dominated structure. The results indicate that geological substrate shapes diatom community dynamics primarily through its control of physicochemical water properties. Indicator species analysis (IndVal) identified *Pinnularia subcapitata* W. Gregory as associated with crystalline–metamorphic springs, whereas *Cocconeis euglypta* Ehrenberg, *Cocconeis placentula* Ehrenberg and *Meridion circulare* C.Agardh were linked to carbonate-dominated springs. This study provides one of the first integrative multivariate assessments of crenic diatom communities in the Apuseni Mountains and highlights contrasting ecological patterns associated with different geological settings.

Keywords: Carpathian Mountains, carbonate substrate, metamorphic schist, diatom guilds.

Introduction

Spring ecosystems are characterized by low hydrological variability, hydrochemistry largely determined by the geological substrate, and pronounced microhabitat diversity (Cantonati and Pipp, 2000; Fránková *et al.*, 2009). In mountainous regions, particularly where springs emerge from carbonate or crystalline bedrock, distinct hydrochemical signatures are reflected in parameters such as electrical conductivity and buffering capacity (Ford and Williams, 2007). These environmental properties create crenic refuge habitats that support specialized and often stenotopic biotic communities (Taxböck *et al.*, 2017). The need to study and protect springs is emphasized by their small size and high biotic diversity, as any local disturbance can be detrimental (Fernández-Martínez *et al.*, 2024).

Diatoms (phylum Bacillariophyta) are globally distributed microalgae that occur across most aquatic habitats worldwide, with many taxa exhibiting cosmopolitan distributions (Vanormelingen *et al.*, 2008; Malviya *et al.*, 2016). However, numerous species exhibit narrow ecological tolerances and clear separation along environmental gradients such as conductivity, pH, nutrients or discharge variability (Cantonati *et al.*, 2022; Blanco *et al.*, 2025). Consequently, diatoms are widely used as bioindicators of the ecological status of aquatic ecosystems (Bellinger and Sigee, 2010). In springs, epibryon (epiphytic diatoms living on the surface of bryophytes) and epilithon (taxa growing on the surface of stones) represent specific microhabitat associated communities. Bryophytes provide a complex structural substrate to grow on, with enhanced moisture buffering and microhabitat heterogeneity (Letáková *et al.*, 2018). Often, they host assemblages that differ in composition and diversity from epilithon (Cantonati *et al.*, 2012a). In eucrenal habitats, these diatom communities reflect the distinctive hydrological characteristics of groundwater-fed ecosystems (Cantonati *et al.*, 2012b).

Geological differences, reflected by distinct physical and chemical features, directly influence the diatom assemblages in aquatic habitats (Cantonati 1998; Cantonati *et al.*, 2012a). Shifts in the benthic diatom communities can be observed when hydrochemical factors change the ionic composition or pH stability (Soininen 2007; Fránková *et al.*, 2009). Carbonate spring systems typically exhibit higher alkalinity, greater buffering capacity, and elevated conductivity (Cantonati *et al.*, 2012b) compared to springs emerging from crystalline or siliceous bedrock (Beauger *et al.*, 2023).

Extensive research on crenic diatom communities in the European Alps has employed multivariate ecological modeling to better understand the structure of spring communities (Cantonati *et al.*, 2006; Cantonati *et al.*, 2012b). These studies emphasize the influence of bedrock lithology, discharge regime, and hydrochemical buffering capacity in shaping benthic communities, as demonstrated in karst systems of the Alps and the Mediterranean region (Lai *et al.*, 2020), as well as mineral springs complexes such as Châteldon, Vichy and the Saint-Yorre basins (Baker *et al.*, 2022). Springs are widely recognized as biodiversity hotspots, dependent on continuous input and highly sensitive to climatic and anthropogenic pressures (Angeli *et al.*, 2010; Cantonati *et al.*, 2012a).

In contrast, in the Carpathian Mountains, studies on spring diatoms have largely focused on documenting species composition and exploring ecological gradients in karst and crystalline environments (Fránková et al., 2009; Poulíčková et al., 2005; Micle et al., 2018; Șuteu et al., 2025). Several studies have explored spring typology, hydrological and chemical variability, and crenic phytobenthos structure, contributing with valuable floristic and ecological knowledge from the several mountain regions like the Cracow–Częstochowa Upland and the Western Carpathians (Wojtal and Sobczyk, 2006; Fránková *et al.*, 2009; Hájková *et al.*, 2011; Wojtal and Sobczyk, 2012) and only more recently Romanian Carpathians, specifically the Apuseni Mountains (Micle *et al.*, 2018; Șuteu *et al.*, 2025). However, comprehensive analyses addressing both geological and environmental factors in spring habitats, while addressing functional bioindicator systems, remain limited in this region.

The Apuseni Mountains provide an ideal setting for disentangling these relationships, as contrasting lithological units compose this geographic area. The present study aims to clarify these relationships through an integrated approach that combines community composition analysis with multivariate modelling across contrasting geological substrates. Based on previous research, we hypothesized that geological substrate would explain a significant proportion of variation in diatom community composition due to contrasting hydrochemical profiles. Therefore, we analyzed the crenic diatom communities found in ten springs located in the Apuseni Mountains (Romania) to determine the relative contribution of geology and physicochemical parameters in structuring them. Specifically, we (i) describe community composition and alpha diversity patterns across springs, (ii) assess the extent of community differentiation among geological substrates and (iii) quantify the influence of environmental predictors using multivariate analyses.

Materials and methods

Study area

The sampling campaign included ten freshwater springs located in north-western Romania, within the Apuseni Mountains (Fig. 1). The sampling sites span the Gilău–Muntele Mare Mountain group, the Pădurea Craiului Mountains and the central Apuseni Mountains karst area Padiș–Scărișoara from Bihorului Mountains. This spatial distribution encompasses both karstic and non-karstic hydrogeological settings, allowing comparisons across contrasting geological conditions (Orășeanu, 2016). Hydrologically, the studied springs are associated with three major river basins: the Someșul Rece, the Arieșul Mare (via one of its main tributaries, the Gârda Seacă), and the Crișul Repede (via one of its main tributaries, the Bucea river) (Orășeanu, 2020).

The investigated sites (Tab. 1) represent a range of spring types, from small headwater springs to large karst resurgences, characteristic of the Apuseni Mountain region. Four of the sampled springs are located within the boundaries of Parcul Natural Apuseni, Romania's largest protected karst area: GR4 to GR7. Springs located in the Gilău–Muntele Mare mountain group (MR1, MR2 and MR3) were sampled during July 2017, whereas the sampling of the remaining springs took place in November 2017. Three springs are sampled for the first time regarding their diatom flora: BC8, BC9 and SR10.

The spatial distribution of the investigated springs was mapped in the software R (version 4.5.2) using the packages sf and ggplot2 (Pebesma, 2018; Wickham, 2016). Field obtained spring coordinates were entered as point data in the WGS84 geographic coordinate system and then projected to UTM Zone 34N (EPSG:32634) for map visualization. A base map of Romania was obtained from the Natural Earth dataset (data source Natural Earth, 2024), and spring locations were plotted and symbolized according to geological group.

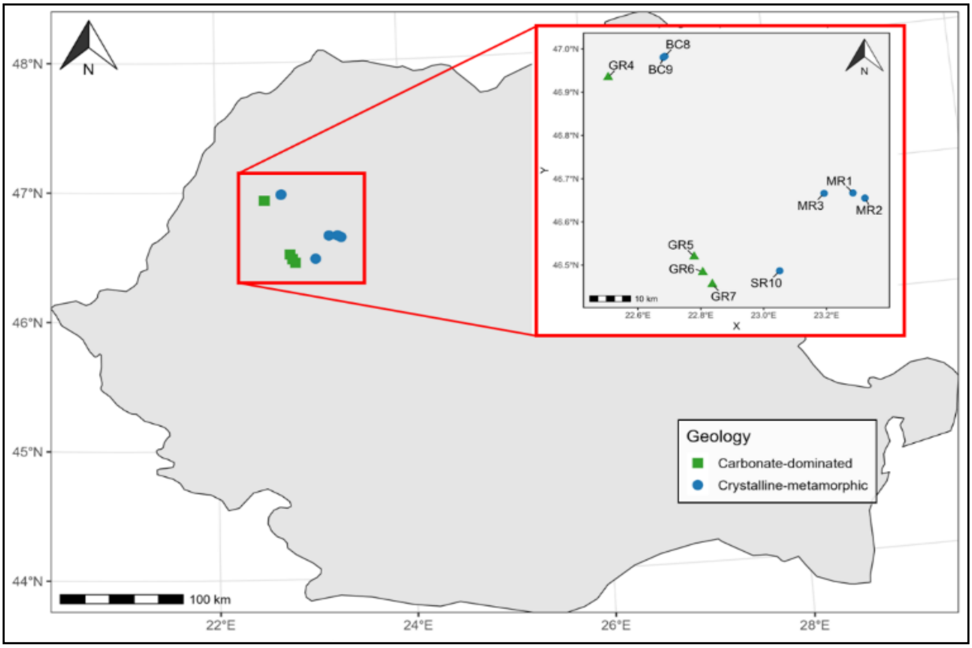

Figure 1. Geographic distribution of the investigated springs in the Apuseni Mountains, Romanian Carpathians (symbols indicate the geological substrate for each site; spring codes correspond to those listed in Tab. 1)

Geological and environmental classifications and assessment

Two geological categories were established based on lithological information obtained from the national geological map of Romania (1:200,000 scale), accessed via the INSPIRE geoportal of the Geological Institute of Romania (data source Geological Institute of Romania, 2018). Geological assignment was verified in QGIS (version 3.44.7) by overlaying spring coordinates onto this layer. For each spring, the lithological unit intersecting the sampling point was identified and used to assign substrate type. Two major geological groups were defined: carbonate-dominated springs, defined as draining limestone and dolomitic formations, characterized by carbonate bedrock and typically associated with higher buffering capacity and mineral content: GR4, GR5, GR6 and GR7; and crystalline–metamorphic springs, defined as draining metamorphic basement rocks (e.g., schists), generally associated with lower mineralization and reduced buffering capacity. The latter group comprised both: crystalline metamorphic (MR1, MR2, MR3, BC8 and BC9) and crystalline - siliceous metasedimentary bedrock (SR10). The applied classification reflects dominant lithology and associated hydrochemical regimes.

Springs were classified according to the crenic typology proposed by Cantonati *et al*. (2007), in: helocrene (the spring rivulet starts where the water emerging diffusely in the seepage area, begin to merge into a small channel), limnocrene (usually a small rim delimiting the spring pool as eucrenal area and the spring stream starts only below this rim) and rheocrene (the spring emerges at the surface through one or more flowing channels). The delimitation of the eucrenal and hypocrenal zones followed the criteria of Cantonati *et al.* (2007), with the eucrenal defined as the area within three meters of the spring emergence point, and the hypocrenal as the area located at least six meters from the same point.

The percentage of shade given by the canopy above the eucrenal area of each site was estimated following the methodology in Șuteu *et al*. (2025). In order to simplify the input of this environmental driver, the percentage values of the shaded area were categorized into three groups: sunny (exposed springs, with up to 25% of shaded areas), half shaded (covered eucrenal area between 26% and 50%) and shaded (from 51 to 100% coverage of the canopy area) (Tab. 1).

Flow intensity was assessed at the spring level using a semi-quantitative, field-based classification. Because comparable quantitative measurements could not be collected at all springs under geomorphological constraints, flow intensity was instead classified into three categories based on visual assessment of water movement: low flow (springs with apparently still water, no visible turbulence of the eucrenal area), medium flow (slow to moderate apparent current velocity), high flow (medium streams with high velocity and high turbulence of the eucrenal area). This categorical variable was assessed by the same person at each sampling site and used to capture ecologically relevant differences in hydrological regime among springs (Tab. 1).

Physico-chemical parameters were measured in situ using portable multiparameter: for pH and conductivity Hanna HI98130 and Hanna HI98194, and YSI-52 for water temperature and dissolved oxygen.

Diatom sampling methodology

At each spring site, two microhabitats were targeted: epilithon (biofilm on submerged stones) and epibryon (diatoms associated with submerged bryophytes). In some springs, additional samples were taken from both the eucrenal and hypocrenal area to account for within-site spatial heterogeneity (Tab. 1). Sampling was restricted to well-developed, spatially extensive microhabitats. At each site, three to five independent subsamples were collected per microhabitat. This increased spatial representativeness and captured small-scale variability. For bryophytes, one tuft was collected per subsample. For epilithon, an equivalent surface of approximately 10 cm^2 area was scraped from a single stone for each subsample. Sampling intensity reflected the local availability of bryophyte substrates. At GR6, two bryophyte taxa were present in sufficient quantity to allow separate sampling, whereas at the other springs diatoms were analyzed from a single bryophyte sample per site.

Table 1. Spring names, codes and associated geological and hydrological characteristic considered for the present study (Abbreviations: K – karst or carbonate-dominated geology; C – crystalline-metamorphic geology; L – limnocrene; R – rheocrene; Hl – helocrene; E – eucrenal area; H – hypocrenal area; Sp - species)

Spring name	Spring code	Sampled microhabitat	Altitude (m)	Mountain group	Geology code	Spring type	Flow class	Shading
Muntele Rece	MR1	Bryophytes (E)	851	Gilău-Muntele Mare	C	L	Low	sunny
Muntele Rece	MR2	Bryophytes (E)	1036	Gilău-Muntele Mare	C	R	Low	shaded
Măguri Răcătău	MR3	Bryophytes (E)	626	Gilău-Muntele Mare	C	R	Low	sunny
Izbucul Izbândișului	GR4	Bryophytes (E, H)	406	Pădurea Craiului	K	L	High	half shaded
Izbucul Tăuzului	GR5	Bryophytes (E) Stones (E, H)	909	Bihorului	K	L	High	half shaded
Izbucul Cotețul Dobreștilor	GR6	Bryophytes (E) Sp1, Sp2 Stones (E, H)	807	Bihorului	K	R	Medium	shaded
Poarta lui Ionele	GR7	Bryophytes (E) Stones (E)	857	Bihorului	K	R	Low	shaded
Bătrânu	BC8	Bryophytes (E)	743	Pădurea Craiului	C	R	Medium	half shaded
Bucea	BC9	Bryophytes (E)	681	Pădurea Craiului	C	R	Low	half shaded
Somesul Rece	SR10	Bryophytes (E) Stones (E)	1521	Gilău-Muntele Mare	C	Hl	Low	sunny

The obtained subsamples from each spring were processed separately to isolate clean diatom frustules using a standard oxidation procedure, applied to both bryophyte and lithic material (Kelly *et al.*, 1998). To ensure sufficient frustule abundance for reliable identification and counting, and to standardize analytical effort across sites, one composite sample was prepared separately for each microhabitat per spring by pooling the corresponding subsamples. Permanent slides were prepared by mounting two drops of homogenized suspension (approximately 0.1 mL) from a 10 mL diatom–water mixture. The identification of algal taxa was made to the species level using the following identification keys: Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b).

Three of the ten springs analyzed in this study (MR1, MR2, MR3) correspond to springs S1, S2, and S3 in Micle *et al.* (2018). For these springs, we used only the raw diatom and physico-chemical data, without reproducing any figures, tables, statistical results, or interpretations from the earlier publication.

Statistical analysis and applied indices

Taxonomic occurrence, defined as the number of taxa present in a sample, was organized in a matrix where 1 indicated the presence of a species in a sample and 0 indicated its absence. The frequency of the identified species was defined as the percentage from the total number of samples explained as: frequent species (a frequency above 80%, i.e., found in more than 8 springs), commonly occurring species (a frequency between 21% and 79%) and rare taxa (frequency of less or equal to 20%, i.e., present in one or two springs). These three categories were used as descriptive parameters throughout the text.

Composite samples representing different microhabitats were treated as separate ecological units because epilithon and epibryon support distinct diatom communities (Poulíčková *et al.*, 2004). Similarity among composite samples was calculated from a presence–absence matrix comprising all recorded diatom taxa, using the Jaccard similarity index in PAST software (version 4.14c; Hammer *et al.*, 2001). In addition, similarity in quantitative community data was assessed using the Bray–Curtis index, computed from the abundance matrix of all the composite samples. Species richness (S), Shannon–Wiener diversity (H′), and Pielou's evenness (J) were calculated for each composite sample using PAST software. Differences in alpha diversity were explored descriptively across geological groups of springs. The Van Dam ecological indicator values (Van Dam *et al.*, 1994) were calculated using OMNIDIA software (version 6.0.9; Lecointe *et al.*, 1993), based on species relative abundances for each sample. The obtained results (salinity preference, oxygen requirements and moisture affinity) were used to support ecological interpretation of assemblage patterns. The following analysis was performed using the RStudio software (version 2026.01.0) and software R (version 4.5.2).

Indicator taxa associated with geological types and microhabitats were identified using the IndVal.g method (999 permutations) from indicspecies package in R software (De Cáceres and Legendre, 2009; De Cáceres, 2023). The ecological guild structure of diatom assemblages was assessed using the DiaThor package (Nicolosi Gelis *et* al., 2022; function *diat_guilds*). Taxa were assigned to ecological guilds and their relative contribution to each sample was calculated based on abundance data. Guilds are separated between: low-profile (small, adnate taxa closely attached to the substrate and tolerant to disturbance), high-profile (stalked or taxa forming colonies which extends into the water column, tolerating only low disturbance), motile (taxa capable of active movement across sediments), planktic (free-floating taxa typical of standing waters), and indeterminate. Guild composition was used to support ecological interpretation of community patterns (Rimet and Bouchez, 2012).

To visualize differences in diatom assemblage composition among composite samples from each spring, composite samples were projected on a non-metric multidimensional scaling (NMDS). Species abundances were first transformed to relative abundances and Bray–Curtis dissimilarity was calculated between composite samples. NMDS ordination was performed in two dimensions ($k = 2$) using the *metaMDS* function in the vegan package (Oksanen *et al*., 2024), with 200 random starts. Stress values were used to evaluate ordination fit. Associations between community composition and environmental variables were explored using the *envfit* function (999 permutations). Continuous variables (temperature, pH, dissolved oxygen, and conductivity), together with ordinal scores derived from canopy cover and flow category, were fitted separately from categorical factors (geology, canopy, and flow) onto the NMDS ordination. This approach allowed testing canopy cover and flow both as ordered gradients and as discrete categories, capturing potential directional trends as well as group-level differences in community composition.

Differences in community composition among geological types were tested using permutational multivariate analysis of variance (PERMANOVA; adonis2, 999 permutations), and homogeneity of multivariate dispersion was evaluated using *betadisper* function from vegan package.

Redundancy analysis (RDA) was conducted using the Hellinger-transformed community matrix to assess relationships between diatom assemblages and environmental predictors. Geological groups (carbonate-dominated vs crystalline–metamorphic) and measured environmental variables (conductivity, canopy cover, temperature, pH, dissolved oxygen) were included as explanatory variables. Categorical predictors were treated as factors. Predictors exhibiting no variation were excluded in the second RDA analysis, which is illustrated in this paper. Multicollinearity among predictors was evaluated using variance inflation factors (VIF), and variables with excessive collinearity were removed where necessary. Model significance was assessed using permutation tests (999 permutations), including: overall model tests, marginal (unique) effects of predictors, axis-specific tests and adjusted R^2 values were calculated to estimate the proportion of explained variation.

To disentangle the relative contributions of geology and hydrochemical variables, variation partitioning (*varpart* function in vegan package) was performed using geology as one explanatory matrix and numeric environmental variables as a second one. Unique and shared fractions of explained variance were interpreted to assess the extent to which geological effects were mediated by environmental gradients.

Results

Qualitative floristic survey

Across the ten studied springs, the diatom community comprised a total of 126 taxa, of which: 54 were identified only in crystalline-metamorphic springs, 26 taxa only in karst springs and 46 were found in both spring groups (Supplementary Table 1). Within the diatom communities identified in this study, a total of 50 genera were recorded. The genera with the highest number of taxa were *Navicula* and *Pinnularia*. Taxon richness varied markedly among sites, ranging from a minimum of 13 taxa in crystalline springs to a maximum of 64 taxa in karst springs. A substantial proportion of the community was recorded only once in the dataset (n=63), representing half of all identified taxa. Some species formed well-developed populations in specific springs (*Humidophilla contenta* (Grunow) R.L.Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot and Kopalová in karst springs or *Tabellaria floculosa* (Roth) Kützing in crystalline-metamorphic springs), whereas other species appeared as a few frustules across samples (*Amphipleura pellucida* (Kützing) Kützing, *Gyrosigma nodiferum* (Grunow) Reimer, *Stephanocyclus meneghinianus* (Kützing) Kulikovskiy, Genkal and Kociolek).

A contrasting pattern was observed for *Achnanthidium minutissimum* (Kützing) Czarnecki, *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot and *Meridion circulare* (Greville) C.Agardh, which occurred in all ten springs, alongside other frequent species like *Cocconeis placentula* Ehrenberg and *Gomphonema parvulum* (Kützing) Kützing. Singleton taxa prevailed in crystalline springs (n=41), with a high number of taxa belonging to genera *Eunotia* and *Pinnularia*, of which 17 taxa were identified in samples from SR10. In karstic springs only 19 taxa were identified with a single occurrence throughout the analyzed samples (i.e. *Gomphonema truncatum* Ehrenberg, *Navicula menisculus Schumann*, *Nitzschia palea* (Kützing) W.Smith).

Community differentiation among springs

Species composition exhibited limited overlap among springs: Jaccard similarity values indicating substantial compositional turnover among springs (Supplementary Figure 1). The crystalline spring SR10 stood out as the most distinctive, sharing only 15% of its taxa with the other springs. The greatest resemblance between the two groups was detected between GR7 and BC9 (43%), both being rheocrene springs.

The relative abundance analysis highlighted *Achnanthidium minutissimum* as the most consistently present taxon, which predominated in six of the 10 springs. This species reached high proportions (up to 68%) and dominated assemblages in crystalline springs, except SR10. The latter exhibited a different dominance pattern with *Eunotia exigua* (Brébisson ex Kützing) Rabenhorst accounting for more than 40% of the samples. Another particular pattern was observed in karst spring GR6, species *Humidophilla contenta* prevailing in both epibryon and epilithon samples. A further taxon capable of attaining dominance and, in some cases, competing with *A. minutissimum* was *Amphora pediculus*, which reached up to 50% relative abundance in GR7 and occurred at lower percentages in several other karst springs. *Planothidium lanceolatum* was also widespread in the dataset and contributed substantial proportions in the crystalline springs (BC8 and BC9).

Diatom diversity (Shannon index) reached a maximum value of 2.04 in samples from karst springs (GR5), where taxon evenness was relatively high (mean 0.58) and dominance low (0.26). Notably, samples from GR6 exhibited the highest dominance index, reflecting the strong prevalence of *H. contenta* on the available substrate relative to other taxa. The diversity of crystalline-metamorphic springs reached a maximum of $H' = 1.93$, despite being represented by a single sample each. Shannon diversity varied only marginally among springs ($F_{10,8} = 2.60$, $p = 0.095$), indicating relatively consistent alpha diversity among springs.

A strong representation of diatom community patterns across the studied springs was provided by a two-dimensional NMDS ordination based on Bray–Curtis dissimilarity (stress = 0.045; Fig. 2). The ordination plot showed a clear tendency for samples to cluster according to geological substrate. Carbonate-dominated springs formed a relatively coherent group, while crystalline–metamorphic springs displayed broader dispersion along the main ordination axes. The high goodness-of-fit values (non-metric $R^2 = 0.998$; linear $R^2 = 0.994$) further confirm that the two-dimensional NMDS solution provides an excellent representation of the underlying dissimilarity structure.

Given the complexity of geological influences on diatom assemblages, measured environmental parameters were projected onto the ordination (Fig. 2). This analysis revealed a strong association between crenic diatom communities and water chemistry. Environmental fitting indicated significant associations between community composition and dissolved oxygen ($r^2 = 0.57$, $p = 0.008$), conductivity ($r^2 = 0.48$, $p = 0.007$), pH ($r^2 = 0.47$, $p = 0.014$), and canopy cover treated as an ordinal variable ($r^2 = 0.59$, $p = 0.002$). Whereas temperature showed a near-significant association ($p = 0.056$), while flow intensity treated as ordinal was not significant ($p = 0.237$). When fitted as a categorical factor, geological substrate was significantly associated with the ordination ($r^2 = 0.195$, $p = 0.029$), indicating compositional differentiation among geological groups.

Results indicate that geological substrate contributes to community differentiation, while hydrochemical and shading-related gradients further structure assemblage variation within and across geological settings.

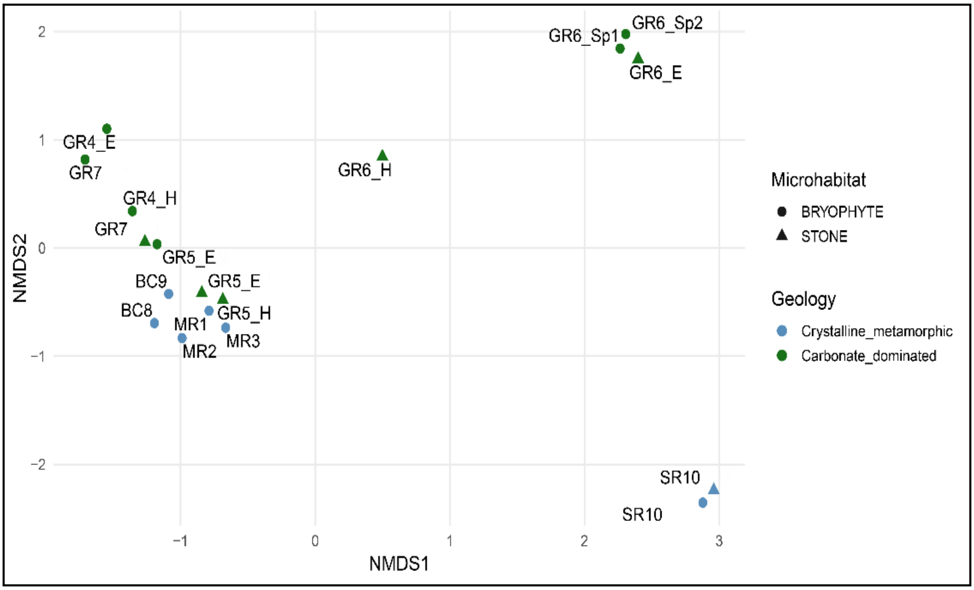

Figure 2. Non-metric multidimensional scaling (NMDS) ordination of diatom communities based on Bray–Curtis dissimilarities (stress = 0.045). Symbol shapes indicate the sampled microhabitat (circles – epibryon; triangles – epilithon) and color indicates geological substrate (green – carbonate-dominated; blue – crystalline-metamorphic). Other abbreviations are provided in Table 1.

Geological and environmental structuring of community composition

Diatom assemblages differed significantly between carbonate-dominated and crystalline–metamorphic springs. In the full dataset, PERMANOVA based on Bray–Curtis dissimilarities revealed a significant effect of geological setting on community composition ($R^2 = 0.138$, $F = 2.56$, $p = 0.02$, 999 permutations). Homogeneity of multivariate dispersion did not differ between geological groups ($p = 0.422$), indicating that the observed differences reflect genuine compositional shifts rather than unequal within-group variability. When tested as a single predictor, geology significantly explained community variation (RDA: $F = 2.64$, $p = 0.03$, 999 permutations; adjusted $R^2 = 8.8\%$), accounting for 14.2% of the total variance. It is consistent with the NMDS clustering pattern and supports a detectable and consistent geological influence on community composition.

Furthermore, an exploratory redundancy analysis (RDA) was conducted using the full set of measured predictors and revealed that community composition was significantly structured by the combined set of geological and environmental predictors, explaining 61.2% of total variance (overall model: $p = 0.002$; adjusted $R^2 = 0.34$). After conducting marginal tests, two predictors remained significant: canopy cover ($p = 0.026$) and pH with a near-significant contribution ($p = 0.059$). Part of the geological effect detected in PERMANOVA was mediated through associated hydrochemical gradients, as indicated by the p-value of 0.141 obtained after accounting for the measured environmental variables. Moreover, variance inflation factors in the full model were below commonly accepted thresholds (all $VIF < 5$), but conductivity and temperature showed overlapping explanatory patterns with pH and canopy, suggesting partial redundancy among environmental predictors. Given the limited sample size relative to the number of predictors, and to avoid over-parameterization, a reduced model was constructed retaining variables which were ecologically interpretable and statistically supported.

Variation partitioning indicated that geological and environmental predictors together explained 23.8% of the variation in diatom community composition (adjusted R^2). Environmental variables accounted for the largest independent fraction (15.0%), whereas geology explained only a small independent component (1.6%), with an additional shared fraction (7.2%) reflecting the indirect influence of geology through hydrochemical gradients.

Although the independent geological fraction was small, the shared component with environmental variables indicates that geological effects on diatom assemblages are mediated through hydrochemical gradients related to bedrock composition.

The reduced RDA model (Fig. 3) including pH, dissolved oxygen, canopy cover, and geology remained significant (overall $p = 0.002$), explaining 52.5% of total variance (adjusted $R^2 = 0.33$). Marginal tests in the reduced model were consistent with the previous outcome, canopy cover ($p = 0.020$) and pH ($p = 0.046$) significantly structured assemblage composition, whereas dissolved oxygen and geology did not retain independent significance. Absence of multicollinearity was confirmed by low values of variance inflation factors.

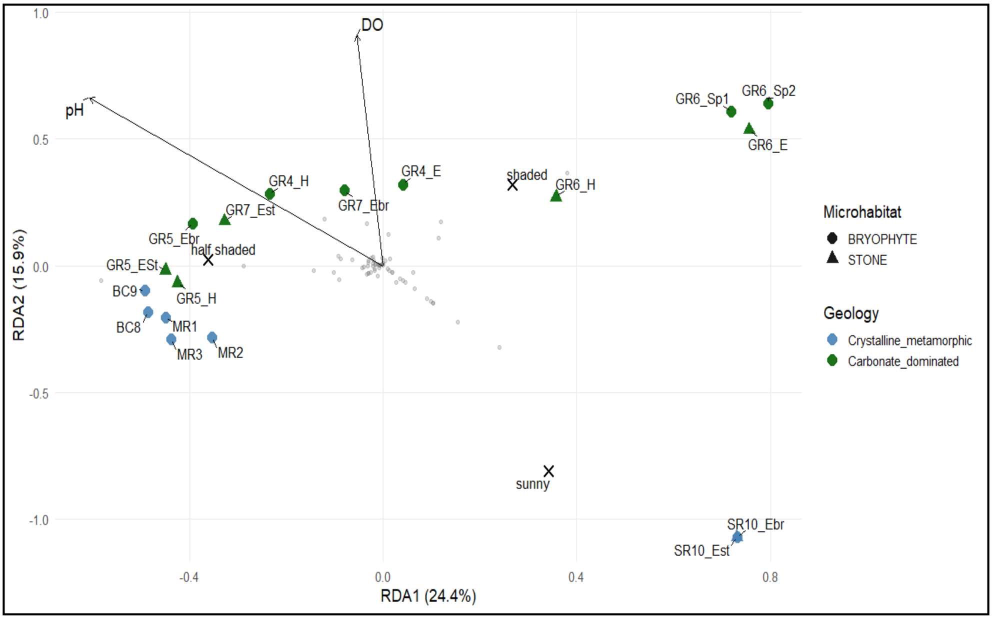

Figure 3. Redundancy analysis (RDA) ordination of diatom assemblages from karst and crystalline springs, based on the reduced model. The first two constrained axes were significant with explained variation for axis 1: 24.4% ($p = 0.010$) and axis 2: 15.9% ($p = 0.013$). Symbol shapes indicate the sampled microhabitat (circles – epibryon; triangles – epilithon) and color indicates geological substrate (green – carbonate-dominated; blue – crystalline-metamorphic), centroid position of canopy cover variable is indicated with x shape. Other abbreviations are provided in Table 1.

Together, these results further reinforce that while geological substrate exerts a detectable influence on community composition, much of its effect is mediated through associated environmental gradients, particularly light availability and hydrochemical conditions (pH), rather than as an independent categorical driver.

Ecological indicator coherence and functional interpretation of assemblage patterns

Across springs in both geological contexts, assemblages were dominated by low-profile taxa, which typically accounted for 65–100% of relative abundance. High-profile and motile guilds varied, but generally remained secondary components, while planktonic taxa were rare. One crystalline–metamorphic spring (SR10) stood out with a distinct configuration dominated by high-profile taxa (80%) and containing notable planktonic forms (15%). This pattern corresponded to its position in the ordination and to its ecological indicator profile, indicating environmental conditions distinct from the other sites. This is consistent with the slightly different crystalline siliceous metasedimentary bedrock and its distinct hydrochemical signature (very low conductivity and comparatively low oxygenation).

Van Dam ecological indicator profiles were consistent with the lithological contrast observed among springs. The pH spectrum coincides with the measured values (7.34-7.69); alkaline indicators representing 71.7% on average, compared with 28.2% neutrophilic indicating taxa. Oxygen preferences were broadly consistent with well-oxygenated conditions characteristic of karst springs with medium to high flow (high-O_2 classes: 73.7%), aligning with the measured hydrochemistry of above 9.4 mg/L of dissolved oxygen. The diatoms sensitive to moisture levels were dominated by taxa classified as "aquatic to aerophilic" (51%) in the carbonate-dominated group, with the highest contribution of aerophilic (21%) and occasionally aerophilic (24.4%) taxa, compared to the opposite group. These values reflect the frequent fluctuations that occur in karst springs, especially at spring GR6, which is known to experience a marked decrease in discharge over the course of the year, sometimes drying out completely for several days (Orășeanu, 2016).

In contrast, crystalline sites displayed a different indicator species distribution. These patterns support geology as an important background factor influencing community composition. In the pH spectrum, communities shifted toward neutrophilic dominance (59.4%) with substantially lower alkaline indicators (25.4%) and a much larger acid-indicator component (maximum of 82.8%). Oxygen preferences remained similar to karst at the group level (78.0% indicating high-O_2 classes). Overall, the karst–crystalline separation is expressed most strongly through the presence of a high percentage of acidophilic taxa and a dominant composition of aquatic to aerophilic taxa (88%).

Indicator species analysis (IndVal.g) identified three taxa significantly associated with the geological groups. *Pinnularia subcapitata* was associated with crystalline–metamorphic springs (IndVal.g stat = 0.655, p = 0.046), whereas

Meridion circulare (stat = 0.905, p = 0.012), *Cocconeis placentula* (stat = 0.795, p = 0.048) and *Cocconeis euglypta* (stat = 0.798, p = 0.039) were associated with carbonate-dominated springs.

These indicator patterns support non-random ecological differentiation consistent with the geological contrast, and coherent with results obtained by environmental fitting analysis and RDA. Furthermore, these results support the hypothesis that geological substrate influences diatom assemblage structure and justify the focused investigation of karst springs conducted in subsequent studies.

Discussion

The species richness identified across the samples collected from the studied crenic sites was high, with a pronounced compositional heterogeneity among sites. Singleton taxa represented half of the total community; their numbers prevailing in crystalline-metamorphic springs. Community similarity between sites was generally low, and ordination analysis revealed a tendency for samples to group according to geological substrate. Multivariate analyses confirmed that diatom assemblage composition differed significantly between carbonate-dominated and crystalline–metamorphic springs. Geological substrate explained a moderate portion of the community variation, while measured environmental variables, particularly light availability and pH, showed significant associations with community structure. Variation partitioning showed that much of the variation attributed to geology was shared with environmental variables, indicating that geology structures diatom communities mainly through associated hydrochemical gradients. Functional analysis revealed low-profile taxa dominated in karstic springs, typical of stable benthic habitats, while high-profile taxa appeared mostly in crystalline-metamorphic springs. Ecological indicator values and indicator species analysis supported the differentiation of communities between geological contexts and highlighted the influence of local hydrochemical and hydrological conditions in both spring groups.

The patterns observed in the present study are consistent with those reported by Cantonati (1998) for mountain springs in the Southern Alps, where diatom assemblages were primarily structured by hydrochemical gradients closely linked to geology. Results show that carbonate springs exhibited higher pH, conductivity and alkalinity, whereas siliceous springs were characterized by lower mineralization and slightly more acidic conditions, resulting in distinct species assemblages. Similarly, our results suggest that geology influences crenic diatom communities mainly through its control on water chemistry rather than acting as a direct ecological filter. In particular, the differentiation of spring groups along conductivity and pH gradients, together with the strong association between conductivity and geological substrate, supports the view that hydrochemical conditions mediate the relationship between geology and diatom community composition. Cantonati (1998) also emphasized that most taxa recorded in springs are widespread rheophilous species typical of cool, flowing headwaters, while strictly crenophilous species represent only a small fraction of the flora. This observation is consistent with our findings, where dominant taxa such as *Meridion circulare* and other common benthic species are characteristic of well-oxygenated, flowing microhabitats frequently associated with bryophytes in spring environments.

Similar findings from Alpine studies identified geology as an important factor contributing to benthic diatom community composition, mainly through its influence on ionic composition and buffering capacity (Aboal *et al*., 1998; Cantonati *et al*., 2006; Cantonati *et al*., 2012b). In crenic habitats, hydrochemical parameters such as conductivity and alkalinity have also been shown to shape diatom communities through the influence of geological substrate (Cantonati and Spitale, 2009). This pattern is further supported by Mediterranean karst springs, where diatom assemblages were strongly associated with mineral content and carbonate geology (Lai *et al*., 2016), and by Beracko *et al*. (2022), who found that water chemistry explained more variation in diatom assemblages than substrate alone. Our findings therefore align with broader European evidence, indicating that geology mainly acts by shaping environmental gradients rather than functioning as a direct ecological filter.

Comparable patterns have been documented in protected areas in the Alps, where hydrochemical contrasts and substratum types are more pronounced (Cantonati and Spitale, 2009). The Mediterranean karst systems similarly show distinct assemblages among springs even within the same geological region (Lai *et al*., 2016). Studies from other regions, including Iranian springs, report that conductivity and nutrient gradients generate clear compositional differentiation at the spring site (Ahmadi Musaabad *et al*., 2019).

Indicator species analysis revealed *Pinnularia subcapitata* as an indicating species of crystalline metamorphic lithological substrate of a crenic aquatic habitat. This species usually supports an interpretation of low buffering and acid influence habitats (Poulíčková *et al*., 2005), which reflects the chemical profile exhibited by the measured variables at the eucrenal area of the crystalline siliceous spring.

Meridion circulare is typically associated with cool, well-oxygenated, flowing microhabitats in spring ecosystems, where it frequently develops on stable substrates and bryophyte-associated niches (Wojtal and Sobczyk, 2012). The species is commonly reported from circumneutral to slightly alkaline waters and moderately mineralized karst springs, conditions often linked to carbonate bedrock influence (Lai *et al*., 2019). In this context, the occurrence and relative abundance of *M. circulare* in our dataset are consistent with the hydrochemical conditions typical of carbonate-dominated springs, supporting the interpretation that geology influences diatom assemblages primarily through its effect on water chemistry and flow-related microhabitat conditions.

Cocconeis euglypta is an adnate, low-profile periphytic diatom, typically associated with stable substrates in well-oxygenated, flowing environments, where its growth form allows it to tolerate moderate turbulence. In spring ecosystems, it is often frequent or abundant across various substrates, including bryophytes, and has been linked to mineral-rich karst springs where hydrochemical gradients and discharge variability influence community structure (Cantonati, 1998). These indicating environmental characteristics are consistent with the habitats in which we identified this species in high abundances.

The frequent occurrence of taxa such as *Amphora pediculus* and *Cocconeis placentula* is consistent with previous studies on karst springs in the Apuseni Mountains, where these species were identified as dominant components of crenic diatom communities (Şuteu *et al*., 2025). *Amphora pediculus*, an alkaliphilous and relatively tolerant species, was identified in our study in moderately to highly mineralized springs and mesotrophic to eutrophic conditions. This taxon alongside the frequent *Cocconeis placentula*, are typically linked to stable lithic substrates in carbonate-influenced systems (Cantonati *et al*., 2012b).

The inclusion of both epilithic and bryophytic microhabitats likely contributed to the relatively high taxonomic richness observed across sites, as these substrates are known to support partially distinct diatom assemblages in spring ecosystems (Cantonati *et al*., 2012a). Site samples differentiation was detectable among springs, but this pattern was largely driven by a small number of ecologically distinct sites, while karst springs shared similar species composition.

The exceptionally low conductivity recorded at Someșul Rece (SR10 = 13 μS cm^{-1}) is consistent with the siliceous metasedimentary bedrock (phyllites and quartzites) of the Pts_Pz unit (a Paleozoic metamorphic lithological unit in the national geological map), which contributes only limited dissolved ions due to the slow weathering of silicate minerals and the absence of carbonate buffering. The diatom community was characterized by a distinct dominance structure driven by *Eunotia exigua*, a well-known acidophilous taxon typically associated with low-conductivity, weakly mineralized waters, reflecting the siliceous substrate and reduced buffering capacity characteristic of crystalline systems. Thus, despite belonging to the same geological group as the other crystalline springs, the distinct community structure observed at SR10 likely reflects locally expressed hydrochemical conditions derived from the siliceous substrate rather than anthropogenic influence or episodic disturbance.

Another similar phenomenon appeared in samples from the karst spring GR7, which exhibited a different ecological signal, dominated by *Humidophilla contenta*. It is a species indicative of intermittently wetted habitats and microhabitats experiencing periodic desiccation or fluctuating moisture conditions (Cantonati *et al*., 2012b; Lowe *et al*., 2014). Its prevalence in both epibryon and epilithon samples suggests localized micro-environmental instability or periodic moisture fluctuations within the spring habitat, consistent with the hydrological instable conditions identified throughout the year at this spring site (Orășeanu, 2016).

Van Dam ecological classification further clarified community structure. Most assemblages were dominated by taxa classified as strictly aquatic or aquatic-to-aerophilic, indicating stable flowing conditions typical of perennial crenic environments. However, the presence of occasionally aerophilic and aerophilic taxa, suggests localized moisture variability and hydrological dynamics. Such taxa have also been associated with fluctuating discharge in Bavarian springs (Cantonati *et al*., 2009) and with intermittent or seasonally variable karst systems (Orășeanu, 2016).

Research on spring diatoms in the Carpathian region has developed more gradually compared with the extensive investigations conducted in the Alps. Existing studies have contributed valuable insights into the diversity and ecology of diatom assemblages in mountain springs and spring fens (Poulíčková *et al*., 2005; Micle *et al*. 2018, Șuteu *et al*., 2025).

Although further research is needed to fully understand the complexity of the Carpathian springs ecosystems, this study provides an initial step toward disentangling the relationships among geology, hydrochemistry, and diatom community composition by integrating environmental data, microhabitat structure, ordination analyses, and ecological indicator approaches. We further contribute to reducing the existing knowledge gap by presenting, for the first time, diatom-based results from three previously unstudied springs.

Conclusions

Diatom communities in the investigated springs were primarily structured by hydrochemical gradients associated with geological substrate, particularly those reflected in conductivity and pH. Multivariate analyses indicated clear differences in community composition between carbonate-dominated and crystalline–metamorphic springs. Although geological substrate contributed to the observed variation in diatom community structure, its influence was expressed mainly through environmental gradients linked to water chemistry. Among the measured variables, pH and canopy cover emerged as important factors associated with differences in diatom community composition, highlighting the combined influence of hydrochemical conditions and local shading on crenic diatom flora.

The ecological characteristics of the recorded taxa and guild composition indicate stable conditions typical of freshwater spring environments, with ecologically meaningful signals of moisture variability and localized nutrient influence. In a broader European context, these patterns are consistent with findings from spring systems in the Alps and Mediterranean mountain region, emphasizing the role of hydrochemistry and geology in shaping diatom communities. At the same time, the results highlight the need for further integrative research on spring ecosystems of the Carpathian Mountains in order to better understand their ecological dynamics and potential responses to increasing environmental pressures.

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Supplementary Table 1. Taxonomic list of diatoms recorded in the studied springs, with presence/absence data across epibryon and epilithon samples from each sampling site. The symbol "–" indicates absence, whereas "1" indicates presence

Taxa	**Author**	**MR1**	**MR2**	**MR3**	**GR4**	**GR5**	**GR6**	**GR7**	**BC8**	**BC9**	**SR10**	**GR5**	**GR6**	**GR7**	**SR10**
		Epibryon										**Epilithon**			
Achnanthidium minutissimum	(Kützing) Czarnecki	1	1	1	1	1	1	1	1	1	1	1	1	1	-
Amphipleura pellucida	(Kützing) Kützing	-	-	-	-	1	-	-	-	-	-	1	-	-	-
Amphora libyca	Ehrenberg	1	-	1	1	1	-	-	-	-	-	1	-	-	-
Amphora ovalis	(Kützing) Kützing	-	-	-	1	1	-	-	-	1	-	1	-	-	-
Amphora pediculus	(Kützing) Grunow	1	1	-	1	1	-	1	1	1	-	1	-	1	-
Aulacoseira alpigena	(Grunow) Krammer	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Aulacoseira nivalis	(W.Smith) J.English & Potapova	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Brachysira brebissonii	R.Ross	-	-	-	-	1	-	-	-	-	1	-	-	-	1
Brachysira vitrea	(Grunow) R.Ross	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Caloneis silicula	(Ehrenberg) Cleve	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Caloneis tenuis	(W.Gregory) Krammer	-	-	-	1	-	-	-	1	-	-	-	-	-	-
Cavinula pseudoscutiformis	(Hustedt) D.G.Mann & Stickle	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Cocconeis lineata	Ehrenberg	-	-	-	1	1	-	-	-	-	-	1	-	1	-

Taxa	**Author**	**MR1**	**MR2**	**MR3**	**GR4**	**GR5**	**GR6**	**GR7**	**BC8**	**BC9**	**SR10**	**GR5**	**GR6**	**GR7**	**SR10**
		Epibryon										**Epilithon**			
Cocconeis placentula	Ehrenberg	1	-	1	1	1	1	1	1	1	1	1	1	1	-
Cocconeis euglypta	Ehrenberg	-	-	-	1	1	1	1	-	-	-	-	-	1	-
Cymbella aspera	(Ehrenberg) Cleve	1	-	-	1	1	-	-	-	-	-	-	-	-	-
Cymbella helvetica	Kützing	-	-	-	-	1	-	-	-	-	-	1	-	-	-
Cymbella lanceolata	C.Agardh	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Cymbopleura naviculiformis	(Auerswald ex Heiberg) Krammer	1	-	-	-	1	-	-	-	-	-	-	-	-	1
Denticula tenuis	Kützing	-	-	-	-	1	-	-	-	-	1	1	-	-	-
Diatoma ehrenbergii	Kützing	-	-	-	-	1	-	-	-	-	1	1	-	-	-
Diatoma moniliformis	(Kützing) D.M.Williams	-	-	-	1	-	-	-	-	-	-	1	-	-	-
Diatoma vulgaris	Bory	-	-	-	-	1	1	-	-	-	-	1	1	-	-
Didymosphenia geminata	Mart.Schmidt	-	-	-	-	1	-	-	-	-	-	1	-	-	-
Diploneis elliptica	(Kützing) Cleve	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Diploneis oblongella	(Nägeli ex Kützing) A.Cleve	-	1	1	-	1	-	-	1	-	-	1	-	-	-
Diploneis ovalis	(Hilse) Cleve	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Encyonema gracile	Rabenhorst	-	-	-	-	1	-	-	-	-	1	1	-	-	1

Taxa	**Author**	**MR1**	**MR2**	**MR3**	**GR4**	**GR5**	**GR6**	**GR7**	**BC8**	**BC9**	**SR10**	**GR5**	**GR6**	**GR7**	**SR10**
		Epibryon										**Epilithon**			
Encyonema minutum	(Hilse) D.G.Mann	1	-	-	1	1	1	-	1	-	1	1	-	1	1
Encyonema silesiacum	(Bleisch) D.G.Mann	-	-	1	-	1	1	-	-	-	-	1	-	-	-
Eunotia bilunaris	(Ehrenberg) Schaarschmidt	1	1	-	-	-	-	-	1	-	1	-	-	-	1
Eunotia exigua	(Brébisson ex Kützing) Rabenhorst	-	1	-	-	-	-	-	1	1	1	1	1	-	1
Eunotia fallax	A.Cleve	-	-	-	-	-	-	-	1	-	1	-	-	-	1
Eunotia glacialis	F.Meister	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Eunotia implicata	Nörpel, Lange-Bertalot & Alles	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Eunotia mucophila	(Lange-Bertalot, Nörpel-Schempp & Alles) Lange-Bertalot	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Eunotia paludosa	Grunow	1	1	-	-	-	-	-	1	-	1	-	-	-	1
Eunotia praerupta	Ehrenberg	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Eunotia tenella	(Grunow) Hustedt	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Fragilaria capucina	Desmazières	-	1	-	-	1	-	-	-	-	-	1	-	-	-
Fragilaria tenera	(W.Smith) Lange-Bertalot	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Fragilariforma virescens	(Ralfs) D.M.Williams & Round	-	-	-	-	-	-	-	1	-	1	1	-	-	1
Frustulia saxonica	Rabenhorst	-	-	-	-	1	-	-	-	-	1	-	-	-	1

Taxa	Author	MR1	MR2	MR3	GR4	GR5	GR6	GR7	BC8	BC9	SR10	GR5	GR6	GR7	SR10
		Epibryon										**Epilithon**			
Frustulia vulgaris	(Thwaites) De Toni	1	-	-	-	1	-	-	1	-	1	-	-	-	-
Gomphonella olivacea	(Hornemann) Rabenhorst	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Gomphonema acuminatum	Ehrenberg	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Gomphonema angustatum	(Kützing) Rabenhorst	-	-	-	-	-	-	1	1	1	-	-	-	1	-
Gomphonema angustum	C.Agardh	-	-	-	-	1	-	1	1	-	-	1	-	-	-
Gomphonema gracile	Ehrenberg	1	-	-	-	-	-	-	-	-	-	1	-	-	-
Gomphonema parvulum	(Kützing) Kützing	1	-	1	-	-	1	-	1	1	1	1	1	1	1
Gomphonema subtile	Ehrenberg	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Gomphonema truncatum	Ehrenberg	1	-	1	-	1	-	-	-	-	-	1	-	-	-
Gyrosigma nodiferum	(Grunow) Reimer	-										1			
Hannaea arcus	(Ehrenberg) R.M.Patrick	-	-	-	-	1	1	-	-	-	1	1	1	-	-
Hantzschia amphyoxis	(Ehrenberg) Grunow	1	-	-	-	1	-	-	1	1	-	-	-	1	-

Taxa	Author	MR1	MR2	MR3	GR4	GR5	GR6	GR7	BC8	BC9	SR10	GR5	GR6	GR7	SR10
		Epibryon										**Epilithon**			
Humidophila contenta	(Grunow) R.L.Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová	-	-	-	-	-	1	-	-	-	-	-	1	-	-
Humidophila perpusilla	(Grunow) R.L.Lowe, Kociolek, J.R.Johansen, Van de Vijver, Lange-Bertalot & Kopalová	-	1	1	-	-	-	-	1	1	-	1	-	1	-
Iconella linearis	(W.Smith) Ruck & Nakov	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Luticola mutica	(Kützing) D.G.Mann	1	-	-	-	1	1	-	-	-	-	1	1	1	-
Melosira varians	C.Agardh	1	-	-	-	-	1	-	-	-	-	-	-	-	-
Meridion circulare	(Greville) C.Agardh	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Navicula capitata	F.E.Fritsch & F.Rich	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Navicula capitatoradiata	H.Germain ex Gasse	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Navicula cari	Ehrenberg	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Navicula cincta	Pantocsek	-	-	-	1	1	1	-	-	-	-	1	-	-	-
Navicula cryptotenella	Lange-Bertalot	1	1	-	1	1	1	-	-	-	-	1	1	1	-
Navicula gregaria	Donkin	1	1	-	-	-	-	-	-	-	-	-	-	-	-
Navicula menisculus	Schumann	-	-	-	-	1	-	-	-	-	-	1	-	-	-
Navicula phyllepta	Kützing	-	-	-	-	1	-	-	1	-	1	1	-	-	1

Taxa	**Author**	**MR1**	**MR2**	**MR3**	**GR4**	**GR5**	**GR6**	**GR7**	**BC8**	**BC9**	**SR10**	**GR5**	**GR6**	**GR7**	**SR10**
		Epibryon										**Epilithon**			
Navicula radiosa	Kützing	1	1	-	1	1	-	-	-	-	-	-	-	-	-
Navicula recens	(Lange-Bertalot) Lange-Bertalot	-	-	-	-	1	-	-	-	-	-	1	-	-	-
Navicula rhyncocephala	Kützing	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Navicula rostellata	Kützing	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Navicula tripunctata	(O.F.Müller) Bory	-	-	-	1	1	1	1	-	1	-	1	1	1	-
Navicula trivialis	Lange-Bertalot	-	-	-	-	1	-	-	-	-	-	1	-	-	-
Navicula viridula	(Kützing) Ehrenberg	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Neidium alpinum	Hustedt	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Neidium ampliatum	(Ehrenberg) Krammer	-	-	-	-	-	-	-	1	-	1	-	-	-	1
Neidium bisulcatum	(Lagerstedt) Cleve	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Neidium dubium	(Ehrenberg) Cleve	-	1	-	-	-	-	-	-	-	1	-	-	-	-
Nitzschia dissipata	(Kützing) Rabenhorst	1	-	-	-	1	1	-	-	-	-	1	-	-	-
Nitzschia intermedia	Hantzsch ex Cleve & Grunow	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitzschia microcephala	Grunow	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Nitzschia palea	(Kützing) W.Smith	1	1	1	-	1	-	-	1	-	-	1	-	-	-
Nitzschia paleacea	(Grunow) Grunow	1	-	-	-	1	-	-	1	-	-	-	-	-	-
Nitzschia perminuta	Grunow	-	-	-	-	-	-	-	1	1	-	-	-	-	-

Taxa	**Author**	**MR1**	**MR2**	**MR3**	**GR4**	**GR5**	**GR6**	**GR7**	**BC8**	**BC9**	**SR10**	**GR5**	**GR6**	**GR7**	**SR10**
		Epibryon										**Epilithon**			
Odontidium hyemale	(Roth) Kützing	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Odontidium mesodon	(Ehrenberg) Kützing	1	1	-	1	1	-	1	1	1	-	1	-	-	-
Pantocsekiella ocellata	(Pantocsek) K.T.Kiss & Ács	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Pinnularia abaujensis var. linearis	(Hustedt) R.M.Patrick	1	1	-	-	-	-	-	-	-	-	-	-	-	-
Pinnularia biceps	W.Gregory	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Pinnularia borealis	Ehrenberg	-	1	-	-	-	-	-	-	-	1	-	-	-	1
Pinnularia divergens	W.Smith	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Pinnularia divergentissima	(Grunow) Cleve	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Pinnularia interrupta	W.Smith	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Pinnularia isselana	Krammer	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Pinnularia legumen	Ehrenberg	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Pinnularia media	(Krammer) Kulikovskiy, Lange-Bertalot & Metzeltin	-	-	-	-	-	-	-	-	-	-	-	-	-	1

Taxa	**Author**	**MR1**	**MR2**	**MR3**	**GR4**	**GR5**	**GR6**	**GR7**	**BC8**	**BC9**	**SR10**	**GR5**	**GR6**	**GR7**	**SR10**
		Epibryon										**Epilithon**			
Pinnularia microstauron	(Ehrenberg) Cleve	-	-	-	-	1	-	-	-	-	1	-	-	-	1
Pinnularia nodosa	(Ehrenberg) W.Smith	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Pinnularia obscura	Krasske	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Pinnularia rupestris	Hantzsch	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Pinnularia subcapitata	W.Gregory	-	1	-	-	-	-	-	1	-	1	-	-	-	1
Pinnularia sudetica	Hilse	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Pinnularia viridis	(Nitzsch) Ehrenberg	-	1	-	-	-	-	-	1	-	1	-	-	-	1
Placoneis elginensis	(W.Gregory) E.J.Cox	-	1	1	-	-	-	-	-	-	-	-	-	-	-
Planothidium boyei	(Østrup) Van de Vijver, Coomans, Pottiez & C.E.Wetzel	-	-	-	1	-	-	-	1	1	-	-	-	-	-
Planothidium lanceolatum	(Brébisson ex Kützing) Lange-Bertalot	1	1	1	1	1	1	-	1	1	1	1	1	1	-
Rhoicosphaenia abbreviata	(C.Agardh) Lange-Bertalot	-	-	1	1	1	-	-	-	-	-	1	-	-	-
Rhopalodia gibba	(Ehrenberg) O.Müller	1	1	-	-	-	-	-	-	-	1	-	-	-	-
Sellaphora pupula	(Kützing) Mereschkovsky	-	-	-	-	-	-	-	-	-	1	1	-	-	-
Stauroneis anceps	Ehrenberg	-	-	-	-	1	-	-	-	-	1	-	-	-	1
Stauroneis phoenicenteron	(Nitzsch) Ehrenberg	1	-	-	-	-	-	-	-	-	1	-	-	-	-
Stauroneis smithii	Grunow	1	-	-	-	-	-	-	-	-	-	-	-	-	-

Taxa	**Author**	**MR1**	**MR2**	**MR3**	**GR4**	**GR5**	**GR6**	**GR7**	**BC8**	**BC9**	**SR10**	**GR5**	**GR6**	**GR7**	**SR10**
		Epibryon										**Epilithon**			
Staurosira construens	Ehrenberg	-	-	-	1	-	-	-	-	-	-	-	-	1	-
Staurosirella leptostauron	(Ehrenberg) D.M.Williams & Round	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Staurosirella pinnata	(Ehrenberg) D.M.Williams & Round	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Stephanocyclus meneghinianus	(Kützing) Kulikovskiy, Genkal & Kociolek	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Surirella angusta	Kützing	1	-	-	-	1	1	-	1	-	-	1	-	-	-
Surirella elegans	Ehrenberg	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Surirella librile	(Ehrenberg) Ehrenberg	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Surirella microlibrile	Van de Vijver, Pottiez & Jüttner	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Surirella minuta	Brébisson ex Kützing	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Tabellaria flocculosa	(Roth) Kützing	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Tabularia fasciculata	(C.Agardh) D.M.Williams & Round	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Ulnaria ulna	(Nitzsch) Compère	-	-	-	-	1	1	-	-	-	-	1	1	-	-

Supplementary Figure 1. Cluster analysis of spring samples based on Jaccard similarity, illustrating patterns of resemblance in diatom species composition among the investigated springs.