Diatom Species Richness in Swiss Springs Increases with Habitat Complexity and Elevation

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Abstract: Understanding the drivers of species richness gradients is a central challenge of ecological and biodiversity research in freshwater science. Species richness along elevational gradients reveals a great variety of patterns. Here, we investigate elevational changes in species richness and turnover between microhabitats in near-natural spring habitats across Switzerland. Species richness was determined for 175 subsamples from 71 near-natural springs, and Poisson regression was applied between species richness and environmental predictors. Compositional turnover was calculated between the different microhabitats within single springs using the Jaccard index based on observed species and the Chao index based on estimated species numbers. In total, 539 diatom species were identified. Species richness increased monotonically with elevation. Habitat diversity and elevation explaining some of the species richness per site. The Jaccard index for the measured compositional turnover showed a mean similarity of 70% between microhabitats within springs, whereas the Chao index which accounts for sampling artefacts estimated a turnover of only 37%. Thus, the commonly applied method of counting 500 valves led to an undersampling of the rare species and might need to be reconsidered when assessing diatom biodiversity.

Keywords: rare species; biodiversity; microhabitats; environmental determinants

1. Introduction

Understanding the factors that drive gradients of species richness is one of the major challenges of ecological and biogeographical research. To investigate patterns of species richness and the related environmental drivers, elevational gradients have received considerable attention in the last decade, and have become firmly established as a replicable methodology [1–4]. Elevational richness patterns across a wide range of taxa can show a variety of response shapes to the gradient, from monotonic declines to more or less hump-shaped patterns, with maximum richness at some intermediate point of the gradient [3–5]. For microorganisms, patterns of species richness along elevational gradients can, however, contrast with those of macroorganisms [6] and the determinants of elevational distributions of microorganism species richness are still poorly understood. Studying microorganisms along elevational gradients might therefore provide important insights into the processes determining species richness along elevational gradients. Moreover, different aspects of biodiversity can be driven by different main factors: Wang et al. [7] for instance, found for stream diatom assemblages along elevational gradients

in Asia and Europe that richness was mostly related to pH, while evenness was mostly explained by total phosphorus.

Diatoms are an important group of microorganisms, being one of the most diverse groups of single-celled algae that are distributed worldwide in nearly all aquatic ecosystems. They show a high variation in ecological requirements and are characterized by an outer silica frustule that stays preserved over long periods of times [8]. These characteristics make them valuable environmental indicators in studies of climate change, acidic precipitation, and water quality [9]. Additionally, diatoms are important primary producers in aquatic ecosystems, with an estimated share of up to 20% of the worldwide primary production [10]. Despite their valuable contributions to ecosystem functioning and their value as ecological indicators, the distribution and drivers of diatom diversity remain poorly understood. Diatoms were considered to be cosmopolitan, with only little dispersal limitation [11,12]. Their high dispersal abilities lead to a strong connection between the environment and species composition and diversity, since species can easily reach favorable habitats. The statement of cosmopolitism, however, has become controversial, due to increasing evidence of endemic species [13–17] as well as the problem to identify environmental drivers for diversity and distribution. Moreover, using combined datasets covering very large spatial scales, several studies have shown that population divergence in diatoms apparently takes place over distances of 100 s to 1000 s of kilometers, and that dispersal-related factors generally fail to explain part of the variation in the diatom community structure, with the exception of datasets that span more than 2000 km [18,19]. Studying different ecoregions in Finland in a study area with a diameter of about 300 km, Teittinen and Soininen [20] showed for diatom assemblages in springs that spatial factors were of minor importance as compared to environmental filtering. By studying elevational patterns of different microbial groups (including diatoms) in subarctic ponds, Teittinen et al. [21] confirmed that aquatic autotrophs are primarily controlled by environmental filtering.

In springs, where diatoms are often the most important primary producers, diatom diversity can be influenced by a variety of environmental factors. Water current velocity and the availability of semiaquatic habitats are probably the most important environmental variables driving species richness for flowing and seepage springs [22–24]. Additionally, many studies, including Cantonati et al. [25] for flowing springs of the south-eastern Alps, and [26] for spring fens of the western Carpathians, showed that pH, conductivity, elevation, nitrate, sulphate, and total phosphorus are important environmental determinants of diatom richness. Additionally, calcium ion concentration and nitrate concentrations can affect diatom species richness as shown for anthropogenically altered springs in Poland [27]. A common point of all studies investigating environmental drivers of diatom diversity is that all these studies revealed that environmental variables can only partly explain diatom diversity.

Yet, biodiversity is not only about species numbers but also about spatial changes in species composition. Compositional turnover can be influenced by a variety of factors including stochastic (random) and deterministic processes of community assembly [28–32] as well as species pool sizes [17], and hence, by factors that vary at large extents and trickle down to the local scale [33]. Such factors include habitat area [34], the evolutionary history of lineages and regions [35–38], the cumulative effects of stochastic variation [39], or sampling constrains [40–44]. The most important factors influencing compositional turnover of diatom communities are presumably geogenic variables: pH, conductivity, alkalinity [7,21,45]; hydrological stability [22,24,45]; substrate-particle size, light availability, temperature, nitrate, and phosphorus [7,22,25]; or substrate types [25,46].

Finally, the perception of all these patterns is also influenced by sampling strategy. In particular, since typically in any biotic community a few species are common while most species are rare, incomplete sampling will lead to underestimation of total species numbers and as well to biases in the calculation of community similarity between sites [47]. These biases can be countered by a number of statistical approaches, e.g., [48], but their influence on our understanding of patterns of diatom diversity remain poorly explored.

Here, we investigated changes in species richness and turnover in spring diatom communities in near-natural springs along an elevational gradient in the Swiss Alps. The aims of the study were to answer the following questions: What is the elevational pattern of diatom richness in springs in the Alps? Which environmental variable is the most influential on species turnover between the habitats? Does restricted sampling lead to higher similarities between habitats due to the undersampling of the communities natural to each habitat, or does it lead to lower similarity between habitats, as despite communities being sufficiently sampled, rare species are missing from the sample? We therefore aimed to investigate the influence of sampling constrains on environmental determinism on turnover rates along an altitudinal gradient to move a step forward in disentangling the drivers of diatom community composition.

2. Materials and Methods

2.1. Study Area and Sites

Sampling was undertaken in 71 near-natural springs across Switzerland at elevations from 520 m to 2527 m a.s.l. (Figure 1) and covering different spring types. We distinguished between rheocrenes ($n = 29$; flowing springs), rheohelocrenes ($n = 10$; seepage springs on steep slopes), helocrenes ($n = 15$; seepage springs), limnocrenes ($n = 1$; pool springs), linear springs ($n = 3$; seepage springs with a fluctuating outflow, e.g., depending on precipitation), hygropetric springs ($n = 2$; seepage springs flowing over rocks), and walled springs ($n = 6$; altered springs with a rheocrenic character, e.g., water catchment, outflow through pipes, but the springs remains mostly in a near-natural character). Near-natural springs are optimal study systems for many groups of organisms [49–52] including diatoms [53] since they cover a variety of microhabitats on a small spatial scale, show only limited anthropogenic disturbance, and often reveal high species richness, e.g., [25,50]. Permanent springs are known to be stable habitats, since a number of environmental factors are determined by the aquifer feeding the spring [54]. However, springs can vary greatly among each other, even at a local scale, in habitat diversity and physical and chemical parameters, e.g., [50].
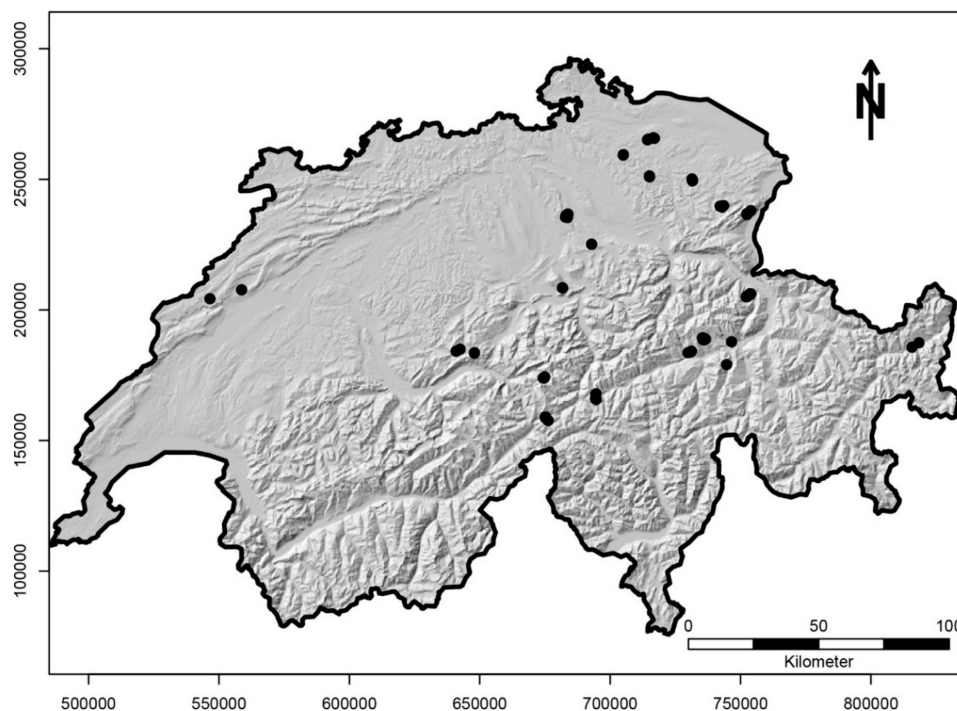

Figure 1. Locations of the springs sampled in Switzerland ($n = 71$). Some sites were in close proximity; therefore, points overlap.

2.2. Field Sampling

We defined five different microhabitats that were sampled if present in each spring: stones, sediment, bryophytes, leaf litter, and filamentous green algae (Table S2). We collected 175 samples in 71 springs. From every habitat, we sampled periphyton to determine species richness and community composition in a minimum of three different spots within a spring. Stones offer a steady habitat to benthic diatoms, can vary in size, and are usually exposed to the running water. Sampling of the stone habitat was conducted by sampling 5–10 stones in each spring (depending on their size), or if only small gravel (ø < 2 cm) was available, 10–15 small stones were taken as a whole. Sediment is irregularly distributed in springs and is often accumulated in areas of still water. Sediment consists mainly of sand and silt but also organic detritus. Sampling of the sediment habitat was conducted by taking circa 10–20 cm^3 of sediment from the surface. Bryophytes build canopies along the spring's edges or around the spring's mouth over the whole spring diameter. On these bryophytes, epiphytic diatoms are found while among the stems and leaves also planktic diatoms can be trapped. Filamentous green-algae build small patches of densely growing filaments, offering epiphytic diatoms a habitat. Leaf litter are dead leaves fallen from the trees around the spring. Epiphytic or diatoms with low light requirements can be found there. Bryophyte habitat, filamentous green-algae habitat, and leaf litter habitat was sampled by taking between 30–40 cm^3 of each habitat as a whole.

Sampling was conducted during the summer months 2009–2011, and every spring was only sampled once. In walled springs, rheocrenes, and rheohelocrenes, spring area was delimited where bryophyte canopies stopped growing. Helocrenes and hygropetric and linear springs were sampled on a stretch of maximal 3–5 times the spring mouth diameter, and the limnocrene was delimited by the pool's surface size.

2.3. Laboratory Analyses

Samples were preserved in a 4% formaldehyde solution. Samples from calcareous bedrock, small gravel (ø < 2 cm), bryophytes, filamentous green-algae and leaf litter were treated with hydrochloric acid to remove carbonates and to detach the adhering diatoms. The cleaned substrates and larger remaining particles were filtered out with a small meshed sieve. Diatom frustules were cleaned from organic matter in concentrated sulphuric acid and potassium nitrate, followed by washing with deionized water [55]. Cleaned diatom frustules were mounted on microscope slides with Naphrax (refractive index = 1.74), two permanent slides were mounted per sample. Species were identified using species descriptions and revisions [56–71].

2.4. Species Richness and Compositional Turnover

We assessed species richness from subsamples (n = 175) by counting 500 valves per slide. We calculated compositional turnover in species communities between microhabitats using two different methods. First, we calculated compositional turnover using the Jaccard index based on the observed species richness from the subsamples of each habitat. The equation for the Jaccard index is computed as 2B = (1 + B) where B is the Bray-Curtis dissimilarity, that is:

$$d_{jk} = \frac{\sum_i |x_{ij} - x_{ik}|}{\sum_i (x_{ij} + x_{ik})}$$

Since our richness data is constrained by a maximum of 500 individuals per sample, we also used the Chao based index for compositional turnover [48] to account for incomplete sampling. This allows us to distinguish if dissimilarities between different microhabitats are solely based on sampling constrains. Chao index tries to take into account the number of unseen species pairs. The function vegdist of the package Vegan in R [72], implements a Jaccard type index defined as $d_{jk} = 1 - U_j U_k = (U_j + U_k - U_j U_k)$, where $U_j = C_j/N_j + (N_k - 1)/N_k \times a_1/(2a_2) \times S_j/N_j$, and similarly for U_k. Here, C_j is

the total number of individuals in the species of site j that are shared with site k, N_j is the total number of individuals at site j, a_1 (and a_2) are the number of species occurring in site j that have only one (or two) individuals in site k, and S_j is the total number of individuals in the species present at site j that occur with only one individual in site k [70].

2.5. Explanatory Factors

We used a set of 10 explanatory factors to describe species richness. Current velocity and shading conditions were estimated according to five-point scales [71]. Conductivity [μS cm^{-1}], pH, dissolved oxygen [%], and temperature [°C] were measured on site using a portable digital probe [Hach-Lange HQ40D including LDO (O_2), PHC (pH, °C), CDC (μS/cm)]. Nitrate (NO_3-N), Total Phosphorus (TP), and Chloride (Cl^-) were measured with Hach-Lange cuvette tests.

2.6. Statistical Analyses

We used Poisson regression to model species richness and predictors and beta regression between compositional turnover and predictors. In the case of high residual deviance after model selection, we used a quasi-Poisson distribution. Diversity as well as turnover can be influenced by several factors. We therefore expected to find combined effects of different independent variables in most cases, thus, we used multiple linear regression with all possible variable combinations to build full linear models. We then applied backward variable reduction to avoid overfitting the multiple regression models. Variables were standardized to zero mean and unit variance to account for different measuring units. To investigate possible drivers of compositional turnover, we used beta regression between the respective turnover index and all 10 environmental variables. To avoid overfitting, we did not perform multiple regressions for compositional turnover, since turnover rates between habitats could only be calculated for a subset of all springs due to habitat occurrence within springs (see *n* in Figure 2. All analyses were conducted using the package 'vegan' [72] and 'betareg' [73] in R 3.0.0 [74].
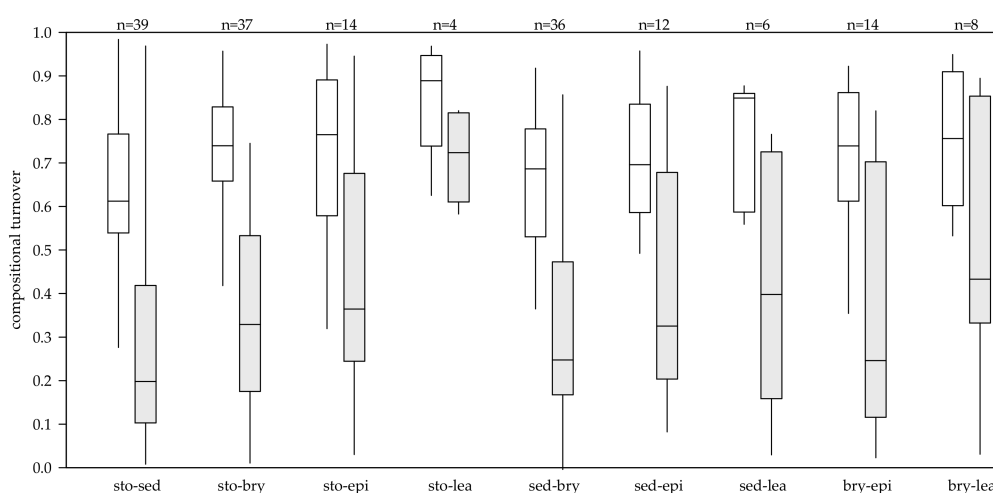

Figure 2. Compositional turnover between different substrates within the same spring calculated with the Jaccard similarity coefficient. White boxes show the similarity between counted samples where only counted abundances were considered. Gray boxes show the similarity between estimated samples where species richness was estimated according using the Chao similarity coefficient. Substrates: Bry = Bryophytes, Sed = Sediment, Sto = Stones, epi = epiphytic on filamentous green-algae, lea = leaf litter, all = cumulative species richness of all substrates at the site. The lower edge of the grey boxes shows the 1st quartile, the upper edge the 3rd quartile, and the line in boxes shows the median.

3. Results

3.1. Habitat Diversity and Species Richness

The most abundant microhabitats within springs were stones ($n = 47$), sediment ($n = 45$), and bryophytes ($n = 57$). In lower numbers, we sampled filamentous green-algae ($n = 17$) and leaf litter ($n = 9$).

On all substrates, we identified a total of 539 diatom species with an average of 55 species per sampled spring. The lowest species richness (five species) was found in a hygropetric spring with a high mineral content, where only stones were available as substrate. The highest species richness (114 species) occurred in a helocrenic spring with four different substrates (Table S1). Means of species numbers per microhabitat were: stones = 39, sediment = 45, bryophytes = 43, leaf litter = 32, epiphytic on filamentous green-algae = 39 (Figure 3).
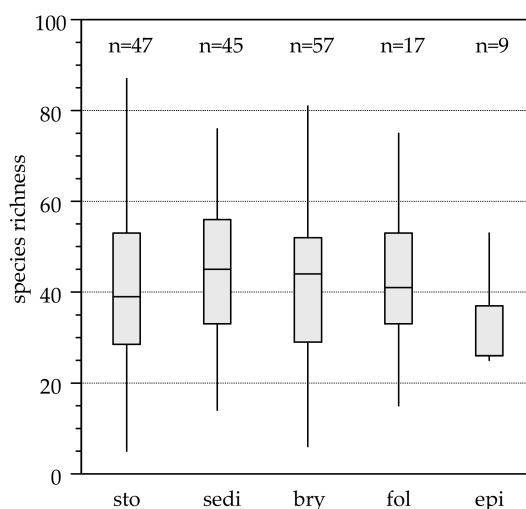

Figure 3. Species richness for each substrate. Mean species numbers per microhabitat were: stones = 39, sediment = 45, bryophytes = 43, epiphytic on filamentous green-algae = 39 and leaf litter = 32. Substrates: sto = stones, sed = sediment, bry = bryophytes, epi = epiphytic on filamentous green algae, lea = leaf litter. The lower edge of the grey boxes shows the 1st quartile, the upper edge the 3rd quartile, the line in boxes shows the median, and the whiskers show the minima and the maxima.

On average, species richness increased monotonically on substrates stones, sediment and bryophytes (Figure 4). The variance, however, was high, resulting in low coefficients of determination.

Table 1. Results of the quasi-Poisson regression and stepwise AIC model selection using environmental factors as independent and species richness as dependent variable. Entry cells are z-values (the regression coefficients divided by its standard error). Statistically significant values are in bold. Species richness = number of diatom species per sampling site, elevation [m], cond = conductivity [μS/cm], temp = temperature [°C], flow = flow velocity and shading in a five-point scale according to Cantonati et al. (2007), P = Total Phosphorus (TP), N = NO_3-N^- [mg/L], Cl = Cl^- [mg/L], HD = Habitat Diversity (number of available substrates) Significance: ° = $p \leq 0.1$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

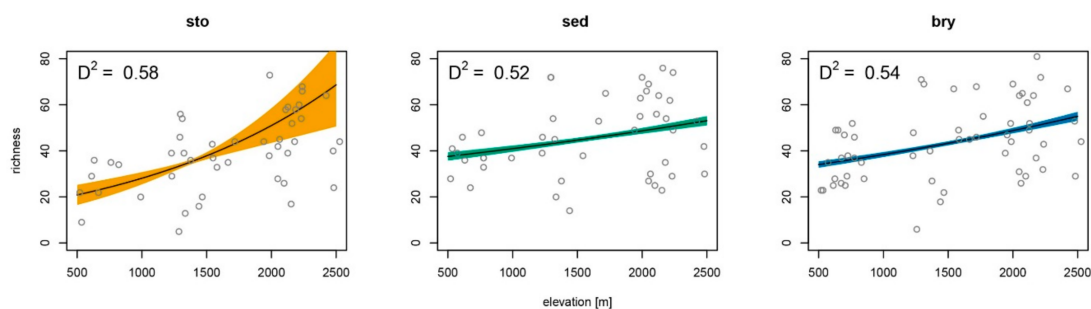| Richness	Elevation	Cond	Shading	pH	Flow	Temp	P	N	Cl	HD	n
S_{all}	2.221 *	−1.543			2.511 *			0.982	1.117	4.953 ***	47
S_{sto}	5.667 ***	3.51 **	3.845 ***	3.027 **		1.439	2.645 *		−1.87		32
S_{sed}	2.188 *	1.777		−1.705			0.968				31
S_{bry}	2.64 *	1.728		−0.788			−0.061				39
S_{epi}				−2.38 *				−1.749	2.194		14
S_{lea}			−3.59 *				6.582 **				7

Figure 4. Species richness of diatoms on different microhabitats along an elevational gradient according to the models presented in Table 1. Microhabitats: sto = stones, sed = sediment, bry = bryophytes.

Habitat diversity (Z = 4.953, $p < 0.001$) was the strongest explanatory factor of species richness per site; weaker explanatory factors were elevation (Z = 2.221, $p < 0.05$) and flow velocity (Z = 2.511, $p < 0.05$) (Table 1). Species richness for single substrates could be explained significantly by elevation for stones (Z = 5.667, $p < 0.001$), with weaker significance for sediment (Z = 2.188, $p < 0.05$) and bryophytes (Z = 2.64, $p < 0.05$). Shading explained species richness significantly for stones (Z = 3.845, $p < 0.001$) and weaker for leaf litter (Z = −3.59, $p < 0.05$). Other environmental variables with a weaker statistical significance were conductivity for stones (Z = 3.51, $p < 0.01$), pH for stones (Z = 3.027, $p < 0.01$) and filamentous green algae (Z = −2.38, $p < 0.05$), and total phosphorus for stones (Z = 2.645, $p < 0.05$) and leaf litter (Z = 6.582, $p < 0.01$).

3.2. Compositional Turnover

For the possible combinations of substrates, the Jaccard similarity of species richness showed a mean similarity between all microhabitats of 70%. The lowest mean compositional similarity, taking all springs into account, was observed between sediment and bryophytes (=65%), the highest mean compositional similarity was found between stones and leaf litter (=84%) (Table 2).

Table 2. Mean compositional turnover between different microhabitats within springs measured using the Jaccard index and the abundance corrected Chao index. Microhabitats: all = all microhabitats within a spring, bry = bryophytes, sto = stones, sed = sediment, lea = leaf litter, epi = epiphytic on filamentous green algae.

	sto-sed	sto-bry	sto-epi	sto-lea	sed-bry	sed-epi	sed-lea	bry-epi	bry-lea	all
Jaccard index	0.65	0.73	0.71	0.84	0.65	0.71	0.76	0.72	0.75	0.70
Chao index	0.30	0.36	0.46	0.72	0.33	0.41	0.42	0.35	0.52	0.37

Measured turnover between microhabitats (sto-sed, sto-bry, sed-bry) showed a monotonic decrease in turnover with elevation, but few of the tested variables explained the compositional turnover between microhabitats. We found no explanation for the compositional turnover between stones and sediment. Between stones and bryophytes, conductivity (Z = 3.101, $p = 0.002$), total phosphorus (Z = 2.518, $p = 0.012$), and chloride (Z = −2.368, $p = 0.018$) were weak explanatory variables. Between sediment and bryophytes, flow velocity (Z = −5.817, $p < 0.001$) and temperature (Z = −3.694, $p < 0.001$) explained significantly the measured compositional turnover, while weaker explanation was provided by conductivity (Z = 2.387, $p = 0.017$), nitrate (Z = −2.156, $p = 0.031$), and total phosphorus (Z = 2.933, $p = 0.003$) (Table 3).

Table 3. Results of beta regression using as dependent variable Jaccard and Chao compositional turnover and environmental variables as predictors. Entry cells are *z*-values (the regression coefficients divided by its standard error). Statistically significant values are in bold. Elevation [m], cond = conductivity [µS/cm], temp = temperature [°C], flow = flow velocity and shading in a five-point scale according to Cantonati et al. (2007), N = NO_3-N [mg/L], P = Total Phosphorus (TP), Cl = Chloride [mg/L], HD = habitat diversity. ° = $p \leq 0.1$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

	Z Value					
	Jaccard			Chao		
	sto-sed	sto-bry	sed-bry	sto-sed	sto-bry	sed-bry
Elevation	−1.587	1.534	−0.313	**−2.581 **	1.213	−1.019
Cond	−0.034	**3.101 **	**2.387 ***	−0.626	**4.200 ***	0.734
Shading	0.55	−0.061	1.63	−0.694	−0.744	1.152
pH	−0.186	−0.853	1.153	−0.373	**−3.383 ***	1.248
Flow	−1.682	−1.573	**−5.817 ***	**−2.798 **	**−2.705 **	**−3.086 **
Temp	−0.209	−0.319	**−3.694 ***	−1.286	−1.457	−1.611
N	−0.358	−0.381	**−2.156 ***	−0.566	−1.789	−1.047
P	0.38	**2.518 ***	**2.933 **	1.014	**4.151 ***	**2.171 ***
Cl	−0.791	**−2.368 ***	−0.021	−0.59	**−3.592 ***	−0.954
HD	−1.01	0.587	−0.936	−1.013	−0.2	−1.766

The Chao similarity index, which accounts for undersampling, showed a mean similarity of 37% between all microhabitats (Table 2). The minimal mean compositional similarity was found between sediment and bryophytes (33%), the maximum between stones and leaf litter (72%). Estimated turnover between microhabitats (sto-sed, sto-bry, sed-bry) showed a monotonic decrease in turnover with elevation, but few of the tested variables explained the compositional turnover between microhabitats. The best explanation for estimated compositional turnover between stones and sediment were elevation (Z = −2.387, *p* = 0.001) and flow velocity (Z = −2.798, *p* = 0.005). Significant explanation between stones and bryophytes was provided by conductivity (Z = 4.200, *p* < 0.001), pH (Z = −3.383, *p* < 0.001), total phosphorus (Z = 4.151, *p* < 0.001), and nitrate (Z = −3.592, *p* < 0.001). Between sediment and bryophytes, flow velocity (Z = −3.086, *p* = 0.002) and total phosphorus (Z = 2.171, *p* = 0.030) were weak explanatory variables for estimated compositional turnover (Table 3).

4. Discussion

Our study revealed relationships between diatom species richness and spring habitat diversity, and environmental variables such as conductivity, flow, light availability and phosphorus, as well as increasing species richness with increasing elevation. At the most general level, our study adds to the evidence that species richness in microorganisms can show contrasting species richness patterns along elevational gradients compared to macro-organisms [7]. Thus, while plants as well as meso- and macrofauna typically show strongly decreasing diversity towards higher elevations in the Alps [75,76] and other mountains [34], we found weakly increasing species richness for diatoms. The lack of a richness decrease, in spite of increasing environmental harshness with elevation, might be due to the "azonal" character of spring habitats that often act in many ways as refugia for different organisms including diatoms [77,78]. Weak elevational richness patterns have also been found in other microorganisms such as bacteria [6]. Other studies on diatom richness have found elevational decreases at low elevation on the Kerguelen Islands [79] and higher elevation in east-central Nepal [80]. By studying elevational patterns of different microbial groups (including diatoms) in subarctic ponds, Teittinen et al. [21] found unimodal elevational patterns for diatoms. Although the elevational pattern of species richness shows an opposing pattern in near natural springs in the Swiss Alps, all diatom studies conducted so far show a high variance in their elevational trends [6,79], pointing towards additional drivers of species richness that might vary independently of elevation, such as habitat diversity and water conductivity.

The number of microhabitats seems to be one of the most important determinants of diatom diversity. Our results in this regard match those of previous studies on diatom substrate specificity from other ecosystems. For example, Smucker et al. [81] in streams in the north-eastern USA missed more potentially occurring species when sampling only stones instead of multiple microhabitats. In boreal rivers in southern Finland, single diatom species showed fidelity to stones, sediments, and plants [82], whereas Cox [46] showed substrate preferences of specific species in German lakes and streams. Species showing substrate preference for stones, wood, sand, and silt were found in rivers across the USA [83]. In Nepalese streams, less than 75% of the total species richness per site was recorded if only one microhabitat was sampled [84]. Cantonati et al. [25] confirmed the relevance of the substrate type as diatom-assemblage determinant in spring habitats. In a global analysis of diatom distribution, Vyvermann et al. [17] showed a connection between local diatom and habitat diversity. The importance of microhabitats for diatom diversity is also underlined by the relatively high rates of turnover between microhabitats within near-natural springs found in our study. Taking into account that undersampling of communities additionally results in underestimating the turnover within diatom communities, the importance of habitat availability as drivers of diatom species richness might still be underestimated.

Focusing on the individual substrates, stones showed the highest statistical support for the relevant environmental determinants. All other substrates only showed limited significance for some of the measured environmental determinants. We hypothesize that stones offer an inert surface and are the most consistent substrate to sample across different spring types, thus resulting in the most standardized data. The other substrates are more variable: sediments are of varying composition of sand, silt, and organic debris, bryophytes are of varying species composition or can accumulate debris, leaf litter is not a permanent substrate, and filamentous green algae are of varying species composition and are not a permanent substrate. Moreover, since sediment usually accumulates in places with still water and since diatom valves and frustules are very resistant to degradation, this substrate integrates parts of different communities that developed over a time span that depends upon the thickness of sediment sampled (taphocoenoses). Accordingly, any underlying relationships with other determinants are probably hidden by the inherent variability of these substrates.

Water conductivity has been detected as environmental determinant of diatom richness and community composition by various other authors in different habitats (e.g., [17,83], in Alpine springs [25], and in carbonate, low-altitude springs [85,86]. Shading or light availability has been mentioned as a relevant environmental driver not only for macroalgae but also for other photosynthetically active organisms [49,50]. Our samples covered a distinct light availability gradient from shaded springs in the forests of the Swiss midlands and the subalpine zone, to the highly light-exposed alpine springs. pH was only recovered as a weak environmental factor for diatom communities on stones. Fránková et al. [26] determined pH as a main environmental variable for diatom species richness, but their studies included very acidic springs in bogs and covered a wider pH gradient.

Although nutrient availability is often mentioned as a driver of diatom diversity [82,83], it played a relevant but secondary role along the studied elevational gradient, as found also in other spring-diatom studies e.g., [25]. Studies revealing nutrients, particularly nitrogen, as important environmental determinants usually cover anthropogenically more intensely impacted areas. Although our datasets comprise springs from some densely populated and intensively used agricultural areas in Switzerland, the water in the aquifers does not transport nutrients, likely because near-natural springs are usually located in areas where groundwater is protected. Lowland springs in Switzerland were all located in forest areas, where the direct impact (e.g., fertilization) by agriculture is small. In contrast, Alpine springs are often affected by cattle-breeding (e.g., cow droppings, trampling damage), but not year-long, and the morphological damages are probably stronger than the impact by nitrate and total phosphorus input.

Our turnover estimates in diatom communities differed by about 30% when we used measured (Jaccard) or estimated (Chao) species turnover. Apparently, the applied method of counting 500 valves led to an undersampling of rare species, leading to an overestimate of community similarity when we only used the measured data (Jaccard similarity). However, it is worth noting that 500 valves counted for each sample is a figure that is equal or higher than that adopted by many diatom studies in Europe (e.g., [87]). Gerecke et al. [88], counting 450 valves per sample, found that diatom species accumulation curves for several springs were still increasing after many years of annual sampling in the frame of long-term ecological research. This suggests that rare species could be of particular interest and that sampling constrains can pose a problem when investigating turnover rates in diatom communities.

When taking environmental factors into account, species turnover between single substrates revealed shading, elevation, and flow rate as important environmental determinants of turnover rates. Yet, only shading was important for the measured species turnover between all microhabitats, although it was less important for the turnover of estimated species composition. For the photoautotrophic diatoms, light availability seems to be an important environmental factor for the dominating species, but for the rare species these factors seem to become more stochastic. Elevation showed some influence on the compositional turnover between stones and sediment, which might be explained with stronger similarities between stones and the sand-dominated sediment than between stones and bryophytes. Turnover rate between stones and sediment and stones and bryophytes was related to water flow rate which seems to select for specific diatom species that do not get washed out of the respective habitat, leading to higher similarity at higher flow rates. Other environmental variables were important only for the compositional turnover of different microhabitat pairs, and were also different for measured Jaccard dissimilarity and estimated Chao dissimilarity. An interpretation might be that the commonly known environmental variables, such as conductivity, pH, flow velocity, and temperature, may select the dominating diatom species on the different substrates, but these variables may not be so important for rare species.

Overall, our results unveiled relationships that are in good agreement with previously shown features: highest species richness in springs on siliceous as compared to carbonate substrate, with siliceous sites located on average at higher elevations than carbonate sites [25]; highest number of diatom taxa in helocrenic springs on siliceous substrate (e.g., [88]); bryophytes have more species as substrate than stones; elevation among the most relevant structuring variables for diatom communities on both stones and bryophytes [25]; geogenic variables (pH, conductivity) as the strongest environmental determinants [21,25]. However, unlike Cantonati and Spitale [22], who could only explain about 3% of the variance with microhabitats, most of our variance was explained by microhabitats. This might be explained by our study which contained five different microhabitats, not only stones and bryophytes.

In relation to conservation purposes and the currently-applied rapid assessment methodologies of diatom richness, problems might arise when using restricted sampling in studies of community composition of diatoms. It is known that rare species are overlooked in limited counting of samples [89], and studies investigating diatom biodiversity without sampling constraint have revealed impressively high species numbers [15] compared to standardized water quality assessment methods. The counting methods applied in water-quality assessment record well known and common species, whereas rare and less distributed species have been neglected thus far. However, there is a potential for the use of rare species for better understanding of ecosystem health [90]. Knowing that species turnover between substrates is underestimated due to sampling constraints, to guarantee more complete floras from oligotrophic environments, diatom biodiversity sampling should additionally focus on rare taxa. It remains unclear why these rare taxa occur at a certain location. Cross contamination was tried to be avoided by cleaning sampling gear between the substrates and the sites. Perhaps these rare taxa are there by chance and disappear within short time slots. Considering how species accumulation curves become flatter with increasing sampling, it is perhaps necessary to count several thousand valves to get a standardized definition of the term "rare taxa". This is probably not feasible because it is too time

consuming. The qualitative method of looking additionally a given time, e.g., 10 min, to account as many rare taxa as possible seems to be more feasible.

In conclusion, we found that species richness of diatoms in springs increases with increasing elevation and that habitat diversity and conductivity are important factors for diatom diversity along elevational gradients. The undersampling rather leads to an underestimation in turnover rates by missing rare species from the sample. Turnover also showed an elevational trend, corresponding with the increasing species richness with elevation.

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