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Diatom metacommunity processes in thermo-mineral springs in the Auvergne Region, France

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Data availability statement

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26

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34

Declaration of conflicts of interest

36 The authors declare no conflicts of interest.

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microorganisms

40 **Abstract**

Thermo-mineral springs are specific ecosystems with extreme environmental conditions that constitute
42 interesting models for studying metacommunity processes. Since these springs can be considered as
islands within a terrestrial context, we first aimed to test some predictions of the theory of island
44 biogeography and the distance-decay hypothesis on benthic diatom communities. Then, we aimed to
quantify the influence of physical, chemical, climatic and spatial factors on species assemblages. We
46 evaluated the species-area relationship for all springs and for several group of springs classified
according to their environmental and hydrochemical composition. The influence of environmental
48 variables on α -diversity was tested. We also investigated whether β -diversity was related to spring
isolation or changes in environmental conditions. Finally, we determined the importance of
50 environmental and spatial variables in shaping diatom communities using ordination and variation
partitioning. We did not find any species-area relationship whatever the group of springs investigated,
52 and no geographical distance-decay pattern was observed. We found a significant effect of physical
and chemical parameters on α -diversity and composition. Thus, diatoms communities of thermo-
54 mineral springs did not seem to be influenced by dispersal processes at the scale of our study but
appeared to be patterned by physical and chemical factors.

56

Introduction

58

The equilibrium theory of island biogeography states that species richness depends on habitat size and
60 isolation (MacArthur & Wilson, 1967). Area affects species richness according to the species-area
relationship (SAR), i.e. the larger the sampling area, the greater the number of species found
62 (Arrhenius, 1921; Gleason, 1922). Several processes have been proposed to explain the SAR, such as
the habitat diversity hypothesis (Lack, 1976), passive sampling, or disproportionate effects (Chase et
64 al., 2019, Gooriah & Chase, 2019, Gooriah et al., 2021). Isolation affects communities based on the
dispersal abilities of the species and was formalized, at the community level, by the distance-decay
66 hypothesis (DDR), which assumes that similarity between communities decreases with the distance
between sites (Nekola & White, 1999). The local and regional processes behind these biological
68 patterns have since been integrated within the metacommunity framework providing a more
comprehensive global overview of species assemblages (Leibold et al., 2004).

70 Although such common biological patterns (SAR and DDR) were first described for large
organisms, some studies suggest that similar processes also affect microorganisms (Astorga et al.,
72 2012; Graco-Roza et al., 2022; Heino et al., 2010; Ranjard et al., 2013; Wetzel et al., 2012). However,
the generality of these patterns remains to be tested, as positive SARs have been documented for
74 bacterial communities in aquatic and non-aquatic systems (Bell et al., 2005; Li et al., 2020; Reche et
al., 2005; van der Gast, 2008) whereas both presence and absence of SAR have been reported for
76 micro-algal communities (Bolgovics et al., 2016; Soininen & Meier, 2014; Teittinen & Soininen,
2015, Jamoneau et al., 2022). This calls for additional investigation into the relative importance of
78 both local and regional factors on biological patterns in micro-algal communities.

 Early studies suggested that diatom communities, and more broadly microorganisms, relied
80 only on environmental conditions, in accordance with Baas-Becking's hypothesis: "everything is
everywhere, but the environment selects" (Baas-Becking, 1934). The structure and composition of
82 diatom communities are known to respond to local environmental conditions (Dixit et al., 1992;
Gottschalk & Kahlert, 2012). Current findings also suggest that diatom communities are shaped by
84 both local and regional processes (Göthe et al., 2013; Jamoneau et al., 2018, 2022; Leboucher et al.,
2020; Rimet & Bouchez, 2012; Vilmi et al., 2016). While this statement may hold true for highly
86 connected ecosystems such as streams and lakes, no studies have been carried out on more specific
systems that are strongly spatially isolated, such as thermo-mineral springs (but see Beracko et al.,
88 2022 for an example on rheocrene springs and Teittinen & Soininen, 2015 on boreal springs).

Thermo-mineral spring ecosystems are ecosystems with extreme physical and chemical
90 conditions (e.g. high conductivity, temperature) that are spatially isolated from other aquatic systems.
Thus, they provide a good model for determining the relative importance of regional and local
92 processes in aquatic biological communities. Indeed, such springs can be regarded as isolated
ecosystems within a terrestrial context (Cantonati et al., 2012b), and their biodiversity may thus
94 exhibit patterns similar to those predicted by the island biogeography theory (MacArthur & Wilson,
1967). Although studies have highlighted the importance of environmental conditions in shaping
96 diatom communities in springs (Cantonati et al., 2012a; Sabater & Roca, 1992; Teittinen & Soininen,
2015), no evidence of island biogeography patterns or dispersal related processes have been
98 documented in such communities (Teittinen & Soininen, 2015). Furthermore, these processes have not
been previously explored in environments with harsh environmental conditions, such as thermo-
100 mineral springs. The extreme environmental characteristics of these ecosystems strengthen their
spatial and ecological isolation from other aquatic communities, potentially increasing the relative
102 importance of regional processes related to dispersal limitation in structuring their communities. This
may contrast with other spring communities more closely linked to the hydrological system, both
104 spatially and ecologically.

The Massif Central (France), and especially the Auvergne region, is punctuated by these
106 thermo-mineral springs (see Supplementary Figure 1). Spring water originates from precipitation that
infiltrates the ground due to both gravity and capillary action (Glazier, 2014). The upward movement
108 is driven by hydrostatic pressure and/or gas pressure. It is during the flow through tectonic faults that
these waters acquire their distinct physical and chemical characteristics, including high mineral
110 concentration, elevated temperature, and gas content (Risler, 1974). As groundwater emerges at the
surface, it carries an abundance of mineral elements such as sodium, magnesium, and calcium. This
112 enriched water fosters unique habitats characterized by a halophilic flora (Hugonnot & Ullly, 2003)
and a specific community of benthic microscopic algae (Beauger et al., 2015, 2016, 2019). These
114 ecosystems serve as habitats to rare and endemic species, leading to the discovery of several new
diatom species (Beauger et al., 2015, 2016, 2019, 2022).

116 Thermo-mineral springs of the Massif Central thus provide an exceptional model for
quantifying the relative importance of local and regional processes in structuring diversity patterns of
118 diatom communities. Here, we first tested for a relationship between the area and the number of
diatom species. Second, we expect to find a distance-decay of similarity with geographical distance.
120 Finally, we also hypothesize that diatom community composition also strongly responds to
environmental variables, in particular mineral ion concentrations and temperature, due to the specific
122 environmental conditions of thermo-mineral springs.

Methods

124 Study area

126 The study area encompasses 54 thermo-mineral springs distributed over an area of roughly 1,436 km²
128 (Fig. 1, see Supplementary Figure S1). The distance between pairs of springs ranges from 0.003 to 59
130 km. The study area displays significant climate differences due to its topography. The springs located
132 in the southwestern part of the study area have a mountainous climate with an average annual
134 temperature of 8°C and an average total annual precipitation of around 1000 mm (Staron, 1990). The
136 springs located in the Allier valley have a continental climate with an average annual temperature of
138 11°C and an average total annual precipitation of around 600 mm.

132 Following Risler (1974), thermo-mineral springs considered in the present study were the
134 springs that met at least one of the two following criteria: (i) thermality, i.e. springs whose water
136 temperature at the outlet is higher than the air annual average temperature or (ii) mineralization, i.e.
138 whose total mineralization is between 1 and 6 g/L. Most of the springs investigated met both these
140 criteria, since the high temperature and pressure favor the concentration of mineral salts by dissolution
142 of the bedrock (Risler, 1974). As such, the mineral and thermo-mineral springs have a range of water
144 temperatures from 4°C to 35°C and mineralization from 0.66 to 7.18 g/L.

140 Biological and environmental data

142 Sampling campaigns were carried out between December 2014 and April 2022 depending on the
144 spring. Note that the communities of some springs were monitored for several years, and community
146 composition only marginally vary with time (Millan, 2018, Baker et al., 2022) suggesting a limited
148 influence of sampling year and season on diatom composition (see also Cantonati, 1998, Cantonati &
150 Pipp, 2000). Diatom samples were collected by brushing or scrapping the dominant mineral substrate
152 present at each site (e.g., travertine deposit, small rocks, spring concrete walls, sediments). Raw
154 materials were prepared according to the protocol defined by Prygiel & Coste (2000) for light
microscopy observations. First, a treatment with hydrogen peroxide (H₂O₂, 35%) was applied to
remove organic matter and, if necessary, with hydrochloric acid (HCl 10%) to remove carbonates. The
cleaned material was rinsed several times and subsequently diluted with distilled water to avoid
excessive concentrations of diatom valves on the slides. The identification and counting of diatoms
were carried out on Naphrax[®] mounted slides. Depending on sample density, 300 to 400 valves were
counted per slide with a Leica[®] DM2700M microscope (x1000). Species were identified using several

references (Beauger et al., 2015, 2016, 2017, 2018, 2019, p. 201; Krammer, 2000, 2002, 2003;
156 Krammer & Lange-Bertalot, 1997a, 1997b, 2000a, 2000b, 2004; Lange-Bertalot, 2001; Lange-Bertalot
et al., 2017; Levkov *et al.*, 2016; Wetzel *et al.*, 2015; Wojtal, 2013)

158 Simultaneously, water samples were collected to examine the chemical composition in the
laboratory. The water sample was first filtered using Whatman GF/C filters. The concentrations of
160 lithium (Li^+), sodium (Na^+), ammonium (NH_4^+), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}),
bromine (Br^-), fluoride (F^-), chloride (Cl^-), nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}) and sulphate
162 (SO_4^{2-}) were measured (mg/L). For the cations analysis, a Thermo Scientific Dionex ICS1100 system
was used; and for the anions, a Thermo Scientific Dionex Aquion system. Bicarbonate concentration
164 (HCO_3^-) was determined directly in the field using a HACH Digital Titrator, sulfuric acid (0.1600 N
and 1.600 N), and Bromocresol Green-Methyl Red Indicator (Hach method 8203).

166 In the field, dissolved oxygen measurements (% and mg/L) were made with a Ysi ProODO
probe, along with pH, conductivity ($\mu\text{S}/\text{cm}$) and temperature ($^\circ\text{C}$) measurements using a WTW
168 Multiline P4 probe. The surface area of the springs was determined in the field by matching its shape
to geometric features for which the area formula facilitates its calculation. For example, the surface
170 area of rectangular springs was calculated by multiplying their length and width measurements, while
the surface area of circular springs was calculated using the geometric formula for the area of a circle,
172 with the radius measured in the field. Spring surface areas range from 0.02m^2 to 20.43m^2 . The depth
was measured at several locations to obtain an estimate of the average depth. We estimated the volume
174 of the spring by multiplying the area by the mean depth. Water velocity was measured with a FLO-
MATE model 2000 and light intensity was estimated in the field according to the percentage of
176 vegetation, mineral (for springs spouting in caves) or anthropogenic (built construction above the
spring) covering each spring (a value of 100% indicates that the spring was found in a totally open
178 environment). Geographic coordinates and altitude of the springs were determined with a handheld
DGPS Geo7x (Trimble, Sunnyvale, CA, USA).

180 Climate data were retrieved from the WorldClim database (Fick & Hijmans, 2017). The data
were extracted using the geographical coordinates of the sampling sites at a resolution of 0.5 degree-
182 minutes. Climate variables included mean temperature (ann_temp), maximum temperature of the
warmest month (max_temp), minimum temperature of the coldest month (min_temp), annual
184 precipitation (ann_prec), precipitation of the wettest month (prec_wet) and precipitation of the driest
month (prec_dry).

186

Data analysis

Since the identification of 300-400 individuals does not necessarily provide an exhaustive list of the species richness of a spring (Soininen et al., 2009), we extrapolated the richness values using the Chao1 index (Chao, 1984) which estimates the number of unobserved species based on the number of singletons and doubletons. Statistical analyses of local diversity were conducted using the richness estimated by Chao1 (hereafter species richness S), and the Shannon (H) and Pielou (J) indices.

Environmental variables were transformed to improve normality, except for conductivity, pH, min_temp, light intensity and HCO_3^- , K^+ , Na^+ , Br^- and F^- concentrations. Water temperature, oxygen content (%), water velocity, spring area, spring volume, Mg^{2+} , NO_2^- , NO_3^- , PO_4^{3-} , SO_4^{2-} , altitude and climate variables were log-transformed; Li^+ , NH_4^+ , Ca^{2+} and Cl^- concentrations were square-root transformed.

We first tested the species-area relationship (log-log) for all springs, using the surface-area of the springs. We also tested the species-volume relationship, assuming that the estimated volume of the spring might be a better proxy of the size of the benthic habitat. Next, to minimize any potential environmental impact on this relationship, we tested the species-area and the species-volume relationships for different groups of springs classified with two independent typologies (see Supplementary Figure S2). The first classification separated springs into 2 groups according to their environmental characteristics. This classification was computed with a non-supervised hierarchical classification (method 'complete' in the *hclust* function in R) using an environmental dissimilarity matrix (Euclidean distance) based on all physical, chemical and climatic variables (standardized variables). The second classification separated springs into 5 groups according to their hydrochemistry. To this end, we used a Piper diagram (DIAGRAMMES version 6.59 software, Simler 2018) which represent the chemical facies of a set of water samples with the use of anion (sulfate, chloride and carbonate plus hydrogen carbonate) and cation contents (magnesium, calcium and sodium plus potassium).

To test the influence of environment on local diversity, we computed linear and non-linear models (quadratic regression) with each environmental variable independently. If both models were significant for one environmental variable, the best model was reported based on the analysis of the residuals (Belloto & Sokolovski, 1985). Subsequently, we selected only the significant environmental variables (i.e. quadratic terms for non-linear models) and incorporated them into a multivariate model. Then, we performed a stepwise procedure to derive a final model that preserves the most significant environmental variables. We repeated this pipeline for S (log-transformed), H and J . Finally, we assessed potential model misspecification by conducting Moran's I tests on the residuals of the models to examine any spatial autocorrelation (Dormann, 2007).

222 For all subsequent analyses, collinear environmental variables were eliminated using the
Variance Inflation Factor (VIF). Following the recommendations of Borcard et al. (2011), the VIF of
224 every variable was calculated iteratively, removing variables one by one with the highest VIF
exceeding 10.

226 To test the distance decay hypothesis, we constructed a similarity matrix for biological data
(Hellinger transformed β -diversity; Legendre & Gallagher, 2001), and tested its relationship with the
228 geographical distance matrix (Euclidean) with a multiple regression on distance matrices (MRM,
Lichstein, 2007). We also included in the MRM the relationship between β -diversity and physical
230 (water temperature, light intensity, water velocity, surface area and volume), chemical (conductivity,
pH, oxygen content (%) and concentration of Br^- , F^- , Mg^{2+} , NO_2^- , NO_3^- , PO_4^{3-} , SO_4^{2-} , NH_4^+ , HCO_3^- and
232 Ca^{2+}) and climatic conditions (min_temp, prec_dry). All environmental matrices were computed with
the Euclidean distance following variables standardization.

234 The composition of diatom communities was investigated with distance-based redundancy
analysis (dbRDA) based on Hellinger distance. We included both physical, chemical and climate
236 variables as explanatory variables, as well as a set of spatial variables formerly created with Moran's
Eigenvector Maps (MEMs; Dray et al., 2006). MEMs were created following the recommendations of
238 Borcard & Legendre (2002) and Brind'Amour et al. (2018), advocating for the spatial regularization of
spatially irregular sampling sites by creating virtual sites. We thus created two virtual sites, defined as
240 the centroid of the larger areas defined with a Delaunay plot (see Supplementary Figure S3). Only
species that account for at least one percent of the relative abundance of the spring community were
242 included in the dbRDA (Lavoie et al., 2009).

We performed a forward selection with a dbRDA computed for each group of variables to
244 include only significant variables (forward selection with double-stopping criterion following Blanchet
et al., 2008). The retained variables were conductivity, pH, and concentrations of Ca^{2+} , HCO_3^- and F^-
246 for chemistry. Additionally, water temperature and spring volume were considered for physical
attributes, while climate variables included precipitation of the driest month. Spatial variables were
248 represented by eight MEM axes. Finally, we used variation partitioning to investigate the relative
influence of chemical, physical, climatic, and spatial variables on diatom communities.

250 All statistical analyses were performed using R statistical software (R Core Team 2016) and
the packages ade4 (Dray & Dufour, 2007), adespatial (Dray et al., 2022) and vegan (Oksanen et al.,
252 2016).

254 Results

256 Drivers of local diversity

258 Species richness ranged from 1 to 48 species depending on the spring (mean=13). No significant
relationship was found between the species richness and the spring area (F-statistic = 1.64, df = 52, P-
260 value = 0.21) nor between the species richness and the volume of the spring (F- statistic = 0.18, df =
52, P-value = 0.67, Fig. 2). Similarly, we did not find any significant relationship between species
262 richness and area or volume when relationships were tested independently for each typological
classification (i.e. two and five groups based on the hierarchical and hydrochemical classifications,
264 respectively).

Out of the 29 variables that were tested in linear and non-linear models, 11 showed significant
266 relationships with local diversity indices (see Supplementary Table S1). It is worth noting that the
relationships with physical variables tend to be linear, whereas the relationships with chemical and
268 climate variables tend to be nonlinear. Following the incorporation of these variables into multivariate
models and subsequent application of a stepwise procedure, only 7 variables were retained as
270 significant contributors to explain diversity indices (see Supplementary Table S2).

All climatic variables were removed during the stepwise procedure. Among chemical
272 variables, the concentration of SO_4^{2-} and NH_4^+ were the two variables explaining species richness (Fig.
3). Species richness peaked at intermediate values of NH_4^+ concentration, and at both low and high
274 values of SO_4^{2-} concentration. Shannon diversity was better explained by phosphate and nitrate
variables (Fig. 3) both evidencing lower diversity at intermediates concentrations. Water temperature,
276 light intensity and water velocity were the main physical variables significantly related to diversity
indices, all of them associated with a decrease of diversity with an increase of their values, except for
278 species richness which peaked at intermediate values of light intensity (Fig. 3). Note that the light
intensity is the only environmental variable influencing the Pielou index. No spatial autocorrelation
280 was detected in the residuals of any of the three models (see Supplementary Table S3).

282 Drivers of β -diversity

284 The relationship between community dissimilarity and geographic distance was not significant
according to the MRM (Table 1). Similarly, no significant relationship was observed between

286 community dissimilarity and dissimilarity in physical and climatic factors (Table 1). However, the
relationship between community dissimilarity and chemical distance is highly significant and positive
288 (Table 1).

290 Drivers of species composition

292 The position of springs on the first two axes of the dbRDA (Fig. 4a) reveals that diatom species
composition was strongly associated with environmental conditions, especially with physical and
294 chemical variables. Specifically, conductivity, ionic compounds and water temperature were
negatively correlated with this axis. At the opposite, three spatial vectors (MEM9, MEM5 and MEM8)
296 were positively correlated with this axis. Axis 2 is also explained by several spatial vectors, calcium
concentration and the volume of springs. Interestingly, the precipitation of the driest month was the
298 sole climate variable retained by the forward procedure and associated with the diatom composition of
thermo-mineral springs in our study area.

300 The major influence of conductivity, ionic compounds and water temperature on diatom
composition of thermo-mineral springs distinguished species specific to halophilic environments and
302 thermo-mineral conditions (e.g. *Crenotia thermalis* (Rabenh.) Wojtal, *Navicula sanctamargaritae*
Beauger, *Halamphora coffeaformis* (C. Agardh) Levkov) from more generalist species (e.g.
304 *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot, *Planothidium straubianum* C.E.
Wetzel, Van de Vijver & Ector, *Sellaphora labernardierei* A. Beauger, C.E. Wetzel & Ector, Fig. 4a).

306 The results of the variation partitioning analysis indicated that 35% of the variability in diatom
community composition could be attributed to distinct sets of variables (Fig. 4b). The chemical
308 parameters were the main factor explaining the variation in diatom composition, representing 10% of
pure effect. Physical, and spatial parameters individually accounted for a comparable proportion of the
310 pure effect on diatom species composition (6-7%), whereas the climate component alone explained a
smaller fraction (4%).

312 Discussion

314 This study explored the patterns of species assemblages within the diatom communities of Auvergne
mineral and thermo-mineral springs. We specifically aimed to test the patterns associated with the
316 island biogeography theory on these communities. We did not find support for a relationship between

species richness and area and any evidence of a geographical distance-decay in similarity. Instead, we
318 found that the factors shaping the composition and diversity of the thermo-mineral spring communities
are mainly related to local environmental conditions.

320

Lack of a species-area relationship

322

The lack of a species-area relationship associated with the absence of the distance-decay pattern in
324 thermo-mineral communities of benthic diatoms suggest that these communities do not conform to the
theory of island biogeography in our study area. This advocates for the absence of passive-sampling
326 due to the low dispersal limitation of diatom over our study area as revealed by previous studies in
different environments (Astorga et al., 2012; He et al., 2020; Teittinen & Soininen, 2015). However,
328 the importance of dispersal varies according to the spatial scale investigated (Verleyen et al., 2009).
The spatial extent of our study area, coupled with the high dispersal ability of diatoms (Kristiansen,
330 1996), may thus be insufficient to evidence any dispersal effect within the thermo-mineral springs of
Auvergne. Additionally, the relatively small size of thermo-mineral springs may lead to a ‘small-
332 island effect’, whereby species richness varies independently of area in small islands (Lomolino &
Weiser, 2001).

334 The species-area relationship is also usually associated with the habitat-diversity hypothesis
(Chase et al., 2019; Lack, 1976) suggesting that the highest species richness is found in larger area due
336 to a higher number of potential habitats. The lack of a species-area relationship supports the idea that
microorganisms in this context depend on habitat heterogeneity rather than habitat size, suggesting
338 that in springs, surface area alone may not be the sole factor influencing habitat heterogeneity
(Cantonati et al., 2012b; Reche et al., 2005; Teittinen & Soininen, 2015). Thus, microhabitat diversity
340 (Cantonati et al., 2012b) as well as the real surface-area of the spring cavity (i.e. the benthic surface)
may have greater influence on species diversity. Although we initially hypothesized that the volume
342 might have partly compensated for the lack of information about the real benthic surface, the absence
of a relationship between richness and volume suggests that this variable is not better than surface
344 water area to capture a potential area-effect due to habitat heterogeneity. In addition, the large size,
complexity, and difficulty of accessing several springs constrained the sampling process, which may
346 not fully represent the heterogeneity among the springs (Teittinen & Soininen, 2015).

The influence of pure spatial effect on species composition found in our results may however
348 indicate the possible role of dispersal processes. Since diatom communities did not seem to be limited
by dispersal over our study area, this spatial structure could be due to high dispersal rates related to

350 mass effect processes (Vilmi et al., 2016), as already suggested for river diatom communities
(Jamoneau et al., 2018; Leboucher et al., 2020). However, the influence of the spatial structure could
352 also reflect a 'hidden' effect of unmeasured, spatially correlated environmental variables. Indeed, the
MEM9 was associated with a gradient of nitrate concentration ($r = 0.43$) and might represent an
354 agricultural spatial gradient.

356 Environmental factors as key drivers of community structure and composition

358 The relationships of alpha and beta diversity with environmental factors are in line with those of
previous studies (Cantonati et al., 2012a; Teittinen & Soininen, 2015) and testifies to the relevance of
360 species sorting as a process driving assemblage patterns. According to our results, the composition and
structure of diatom communities in thermo-mineral springs was influenced by multiple environmental
362 variables. However, water chemistry seems to be the most important component. Local diversity was
influenced by nutrients, in particular nitrogen, phosphate and sulfate concentrations. The quadratic
364 relationships observed in our study between diversity and nutrients partly contrasts with other studies
that demonstrated an increase in diversity with higher nutrient levels (Passy, 2008; Soininen et al.,
366 2016). This indicates more complex processes mediating the relationship between diversity and
nutrients in thermo-mineral springs compared to other freshwater ecosystems. This is likely influenced
368 by the distinctive taxonomic composition of thermo-mineral springs, and the presence of habitat
specialists adapted to these harsh environmental conditions. Indeed, the composition of diatom
370 communities in thermo-mineral spring was greatly patterned by conductivity and ionic components as
previously highlighted in various studies (Passy, 2007; Soininen et al., 2016) and particularly in
372 thermo-mineral springs (Leira et al., 2017, Beauger et al., 2023). Moreover, we might expect an even
stronger effect of environment due to the influence of past environmental conditions on communities,
374 as recently suggested for freshwater diatoms (Viso & Blanco, 2023), although environmental
conditions are relatively stable across seasons in our springs (Millan, 2018).

376 Physical factors also strongly contribute to shape spring diatom assemblages, in particular through
light intensity, temperature and water velocity. Species richness peaked at intermediate level of light
378 intensity in our study, but light intensity was negatively correlated with species diversity indices (H
and J) and was notably the sole environmental factor explaining equitability. This suggests that in
380 open environments, competitive species may dominate the community, while species richness might
be promoted during transitional phases. We also found a negative correlation between water
382 temperature and species richness and diversity, a pattern already found in thermal springs communities

(Delgado et al., 2020) but not necessarily consistent across studies (Leira et al., 2017). In Auvergne, water temperature of thermo-mineral springs appeared to be a critical factor limiting the number of diatom species capable of thriving in such environments. The influence of water temperature on specific composition could be explained by the optimal temperature range for each species, which can lead to a change in species composition upon temperature change (Werner, 1977).

Water velocity was significantly and negatively correlated with species richness. If water velocity is assumed to be a disturbance (Wang et al., 2009), our results suggest that the increasing disturbance preclude some species to settle in the more disturbed habitats, contrary to the results of Baker et al., (2022) in similar ecosystems. Also, we did not find a significant effect of water velocity on species composition in our data, although water velocity is known to influence diatom community composition by removing species that cannot attach to a substrate (Passy, 2007). The low velocity of thermo-mineral springs of our study compared to rivers, where most studies of velocity effects were conducted (Passy, 2007; Wang et al., 2009), may explain the absence of a significant effect on community composition.

We found a minor influence of climate variables on diatom community composition and diversity, considering that this effect may be related to altitude-induced climate variations. The small size of our study area could mitigate the potential influence of climate on species composition, despite the noticeable climate variations between the mountain and valley regions within our study area. In fact, climate variables are known to be of great importance in the β -diversity of river diatom communities at a broader scale (Brittain et al., 2022) and are involved in shaping the composition of diatom communities at the global (Soininen et al., 2016) and at the continental scale (Benito et al., 2018).

Conclusions

The study of the diversity and composition of mineral and thermo-mineral springs communities of Auvergne did not appear to follow the typical ecological pattern of the species-area relationship closely associated with the theory of island biogeography. The specific and heterogeneous conditions of the Auvergne mineral and thermo-mineral springs exert a powerful environmental filter on the diatom communities and primarily shape their composition. Indeed, alpha and beta diversity were mainly influenced by the physical and chemical variables of the water such as temperature, water chemistry and light intensity. Although the lack of influence of geographic distance on beta diversity suggests that diatom communities are not dispersed-limited, a spatial influence was found on the species composition. If such influence does not result from unmeasured environmental gradients, it may be due to regional processes, such as mass effect, governing metacommunities. Thus, as the specific local conditions appear to be the major determinants of the diatom communities of thermo-

mineral springs in Auvergne, these results highlight the need of a site-specific conservation
418 management for this unique flora.

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426 **Author contributions**

Conceptualisation: Pierre Gosseaume, Aude Beauger and Aurélien Jamoneau. Developing methods;
428 data interpretation; writing: all authors. Conducting the research, data analysis, preparation of figures
and tables: Pierre Gosseaume, Aude Beauger and Aurélien Jamoneau.

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690 **Table 1** Multiple regression on distance matrices performed on diatom compositional dissimilarity
 matrix and distance-based ('Euclidean') explanatory variables related to geographical, chemical,
 692 physical and climate

	Coefficients	P-value
Intercept	0.47	1
Geographic	4.10^{-7}	0.613
Chemistry	0.05	0.001
Physics	0.01	0.180
Climate	-4.10^{-3}	0.693
R^2	0.20	0.001

694 **Figures captions**

Fig. 1 Topographic map of the location of the sampled thermo-mineral springs ($N = 54$) in the
696 Auvergne region in France

Fig. 2 Scatter plot of the species richness (estimated with Chao1) and the surface area (a) and the
698 volume (b) of the spring (log-log). Colors represents different class of springs according to their
hydrochemical composition and symbols different class of springs according to their environmental
700 characteristics (see methods)

Fig. 3 (a) Standardized coefficients of multivariate models explaining the species richness (S log-
702 transformed estimated with Chao1, blue), Shannon (H , green) and Pielou (J , red) indices. Error bars
represent the 95% confidence interval. On the right side, scatterplots (b-h) illustrate univariate
704 relationships between diversity indices (S , H , or J) and all the environmental variables integrated in
the multivariate models. Solid lines indicate the fitted regression lines, and dashed lines delineate the
706 95% confidence interval around these lines

Fig. 4 (a) Position of thermo-mineral springs, environmental variables and diatoms species ('+'
708 symbols in purple) on the first two axes of the dbRDA (a). Colors represents different class of springs
according to their hydrochemical composition and symbols different class of springs according to their
710 environmental characteristics (see methods). The MEM variables correspond to the vectors generated
by the MEM analysis. Only the species names of extreme species are displayed and refer to their
712 OMNIDIA code (see Supplementary Table S4). (b) Venn diagram depicting the variance partition of
floristic composition for the different groups of explanatory variables (chemical, physical, climatic and
714 spatial). Only positive values are shown

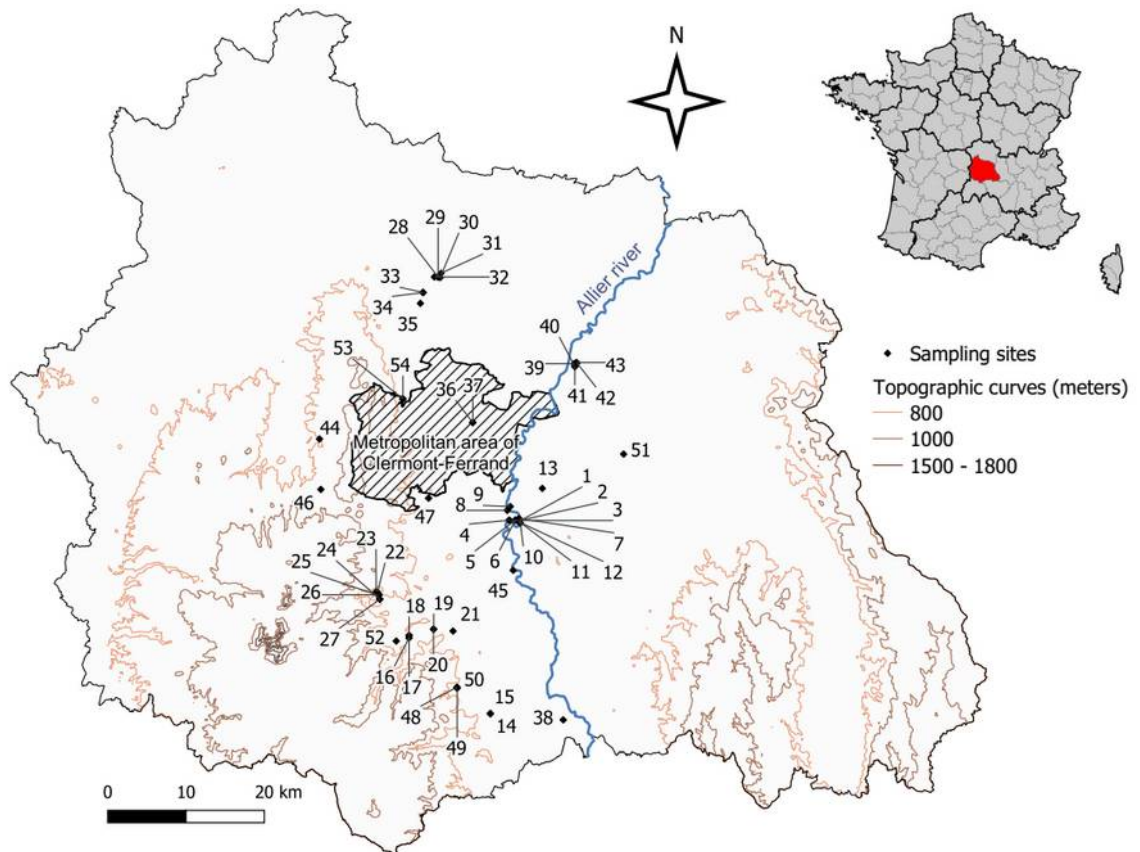


Fig. 1 Topographic map of the location of the sampled thermo-mineral springs ($N = 54$) in the Auvergne region in France

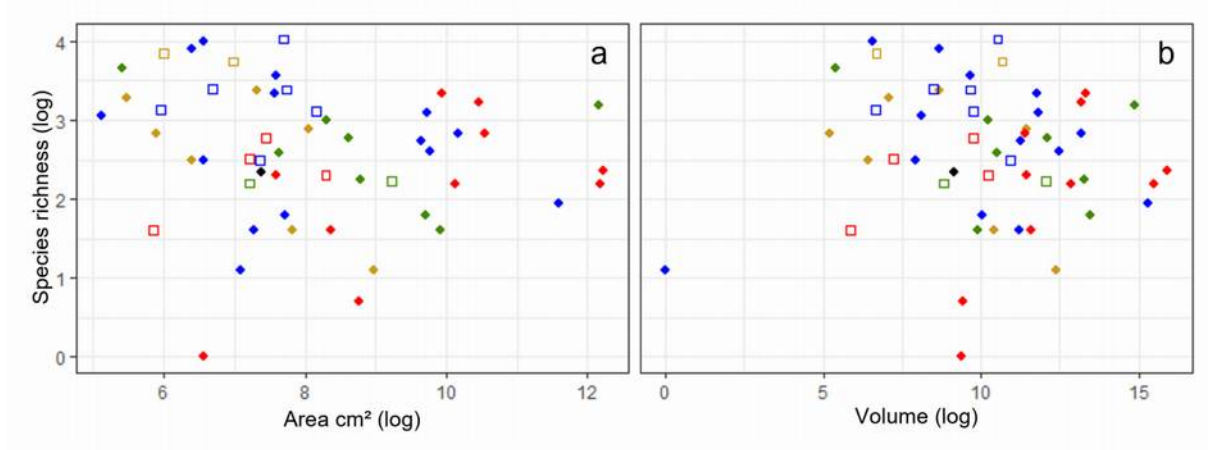
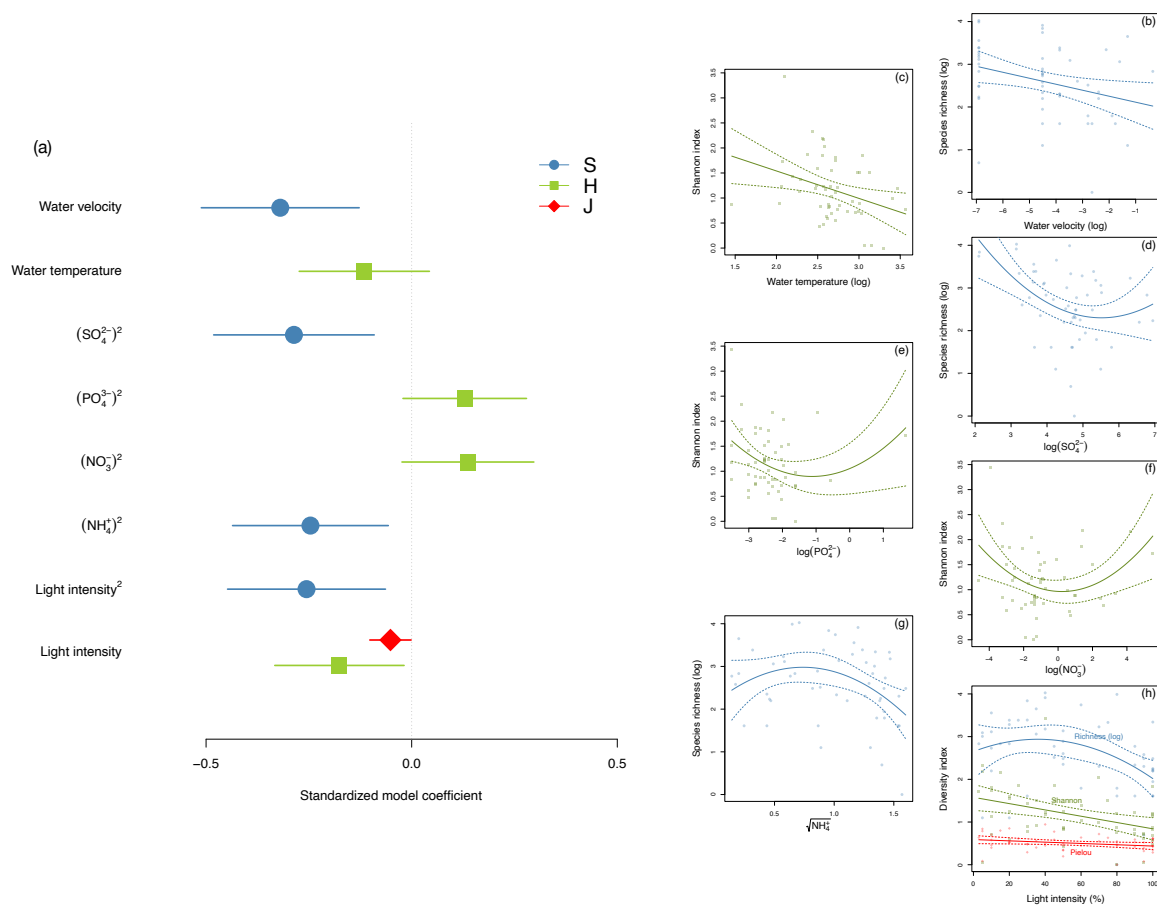
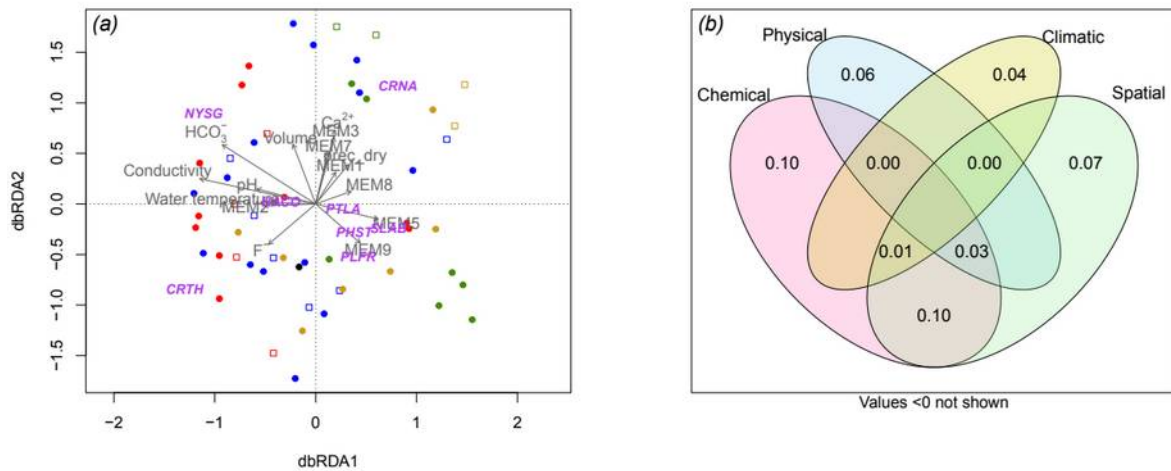


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728 **Fig. 3** (a) Standardized coefficients of multivariate models explaining the species richness (S log-
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736 **Fig. 4** (a) Position of thermo-mineral springs, environmental variables and diatoms species ('+'
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 according to their hydrochemical composition and symbols different class of springs according to their
 environmental characteristics (see methods). The MEM variables correspond to the vectors generated
 740 by the MEM analysis. Only the species names of extreme species are displayed and refer to their
 OMNIDIA code (NYSG – *Navicula sanctamargaritae*, CRTH – *Crenotia thermalis*, HACO –
 742 *Halamphora coffeaformis*, PTLA – *Planothidium lanceolatum*, PHST – *Planothidium straubianum*,
 SLAB – *Sellaphora labernardierei*, PLFR – *Planothidium frequentissimum*, CRNA – *Crenotia*
 744 *angustior*). (b) Venn diagram depicting the variance partition of floristic composition for the different
 groups of explanatory variables (chemical, physical, climatic and spatial). Only positive values are
 746 shown. All environmental fractions are significant (P-value < 0.05)