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► To cite this version:

Lory-Anne Baker, Aude Beauger, Sofia Kolovi, Olivier Voltaire, Elisabeth Allain, et al.. Diatom DNA metabarcoding to assess the effect of natural radioactivity in mineral springs on ASV of benthic diatom communities. *Science of the Total Environment*, 2023, 873, pp.162270. 10.1016/j.scitotenv.2023.162270 . hal-04033005

HAL Id: hal-04033005

<https://hal.science/hal-04033005v1>

Submitted on 16 Mar 2023

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1 **Diatom DNA metabarcoding to assess the effect of natural**
2 **radioactivity in mineral springs on ASV of benthic diatom**
3 **communities.**

4
5 Lory-Anne Baker^{1,2,3}, Aude Beauger^{2,3}, Sofia Kolovi^{3,4}, Olivier Voltaire^{2,3}, Elisabeth
6 Allain^{2,3}, Vincent Breton^{3,4}, Patrick Chardon^{3,4}, Didier Miallier⁴, Céline Bailly⁵, Gilles
7 Montavon^{3,5}, Agnès Bouchez⁶, Frédéric Rimet⁶, Cécile Chardon⁶, Valentin Vasselon⁷, Luc
8 Ector⁸ †, Carlos E. Wetzel⁸, David G. Biron^{1,3} †

9 ¹ Université Clermont Auvergne, CNRS, LMGE, F-63000 Clermont–Ferrand, France ;

10 ² Université Clermont Auvergne, CNRS, GEOLAB, F-63000 Clermont-Ferrand, France ;

11 ³ LTSER “Zone Atelier Territoires Uranifères”, Clermont-Ferrand, France

12 ⁴ Université Clermont Auvergne, CNRS/IN2P3, Laboratoire de Physique de Clermont (LPC),
13 UMR 6533, F-63178 Aubière cedex, France

14 ⁵ Laboratoire SUBATECH, UMR 6457, IN2P3/CNRS/IMT Atlantique/Université de Nantes,
15 4, rue Alfred Kastler, BP 20722, 44307 Nantes cedex 3, France;

16 ⁶ Université Savoie Mont-Blanc, INRAE, UMR CARRTEL, 75 bis avenue de Corzent, FR-
17 74200 Thonon-les-Bains, France;

18 ⁷Science-Management Interface for Biodiversity Conservation (SCIMABIO Interface),74200
19 Thonon-les-Bains, France

20 ⁸Luxembourg Institute of Science and Technology (LIST), Department Environmental
21 Research and Innovation (ERIN), Observatory for Climate, Environment and Biodiversity
22 (OCEB), 4422 Belvaux, Luxembourg;

23
24
25 **Corresponding author:** Lory-Anne Baker,

26 Laboratoire Micro-organismes, Génomes et environnement (LMGE),

27 UMR CNRS 6023,

28 Université Clermont-Auvergne,

29 1 Impasse Amélie Murat,

30 63178, Aubière Cedex, France

31 Mail : l-anne.baker@doctorant.uca.fr

32
33 **Abstract**

34 Little is still known about the low dose effects of radiation on the microbial communities in the
35 environment. Mineral springs are ecosystems than can be affected by natural radioactivity.
36 These extreme environments are, therefore, observatories for studying the influence of chronic
37 radioactivity on the natural biota. In these ecosystems we find diatoms, unicellular microalgae,
38 playing an essential role in the food chain. The present study aimed to investigate, using DNA
39 metabarcoding, the effect of natural radioactivity in two environmental compartments (i.e.
40 spring sediments and water) on the genetic richness, diversity and structure of diatom
41 communities in 16 mineral springs in the Massif Central, France. Diatom biofilms were
42 collected during October 2019, and a 312 bp region of the chloroplast gene *rbcL* (coding for
43 the Ribulose Bisphosphate Carboxylase) used as a barcode for taxonomic assignation. A total
44 of 565 amplicon sequence variants (ASV) were found. The dominant ASV were associated with
45 *Navicula sanctamargaritae*, *Gedaniella* sp., *Planothidium frequentissimum*, *Navicula veneta*,
46 *Diploneis vacillans*, *Amphora copulata*, *Pinnularia brebissonii*, *Halamphora coffeaeformis*,
47 *Gomphonema saprophilum*, and *Nitzschia vitrea*, but some of the ASVs could not be assigned
48 at the species level. Pearson correlation failed to show a correlation between ASV' richness and
49 radioactivity parameters. Non-parametric MANOVA analysis based on ASVs occurrence or
50 abundances revealed that geographical location was the main factor influencing ASVs
51 distribution. Interestingly, ^{238}U was the second factor that explained diatom ASV structure.
52 Among the ASVs in the mineral springs monitored, ASV associated with one of the genetic
53 variants of *Planothidium frequentissimum* was well represented in the springs and with higher
54 levels of ^{238}U , suggesting its high tolerance to this particular radionuclide. This diatom species
55 may therefore represent a bio-indicator of high natural levels of uranium.

56

57 **Keywords:** diatom diversity, mineral springs, naturally occurring radioactivity, *rbcL*,
58 metabarcoding, genetic variants

60 1. Introduction

61 Since the formation of the Earth some 4.5 billion years ago, background radiation levels
62 have varied spatially and temporally in the habitats of the planet (Karam, 2005). For at least 3.5
63 billion years, life has been evolving and adapting in ecosystems with different levels of natural
64 radioactivity (Martell, 1992; Garzón, 2001). Currently, there are regions on the Earth where
65 natural radioactivity can reach very high levels, such as India (Mohanty, 2004), Iran
66 (Khodashenas, 2012) and Brazil (Oliveira, 1994). In Europe, median concentration of uranium
67 in soils is estimated to be around 2 mg.kg⁻¹, with a range of variation from less than 0.1 to more
68 than 50 mg.kg⁻¹ (De Vos et al., 2006). In France, the highest concentrations are found in the
69 Massif Central (a mountain range in the middle of France), where it can reach hundreds of
70 mg.kg⁻¹ in granitic soil (Garnier-Laplace, 2010).

71 In the Massif Central, several mineral springs show significant levels of natural radioactivity
72 (Jacquet, 1929; Boineau, 1972; Risler, 1974; Ielsch, 2017). This natural radioactivity comes
73 from ⁴⁰K and the decay chains of three primordial radioelements ²³⁵U, ²³⁸U and ²³²Th that are
74 naturally present in the Earth's crust, especially in granitic rocks (Métévier, 2001). Because of
75 its longer half-life (4,47 billion years), ²³⁸U and its nuclear decay products (²²⁶Ra, ²²²Rn, ²¹⁰Po)
76 are today the most dominant radionuclides in mineral springs (Eróss et al., 2018; Rihs et al.,
77 2000; Morais et al., 2022). They are α -particle emitters, the most mutagen ionising radiation
78 involved in radioactive stress mainly related to internal irradiation in cells. Thus, mineral
79 springs in the French Massif Central are not only extreme environments due to their significant
80 mineralisation levels, but also their high level of natural radioactivity.

81 Living organisms that inhabit these extreme environments have been evolving and
82 adapting to these conditions. Such mineral springs are therefore interesting 'natural

83 laboratories' in which to study the influence of mineralisation and natural radioactivity on
84 biodiversity. These ecosystems are characterised by short food webs which could be
85 interesting to study the effects of natural radioactivity on primary producers. Diatoms are
86 part of the phytobenthos, and one of the main groups of primary producers in aquatic
87 ecosystems, including mineral springs. Algae are known to be good indicator for
88 radionuclides in aquatic environments and show differences in radionuclides tolerances
89 between species due to their ecological variations (Pradhan, 2019; Banerjee, 2022).

90 The influence of radioactivity on diatoms can be studied at three ecological organizational
91 levels, *viz.* individual, population and community levels (Stevenson, 2014; Pandey, 2017).
92 Gross and Koczy, (1946) (cited in Driver, 1994) reported a severe reduction of diatom survival
93 in a radioactive and experimental medium. In addition, some surveys have already been
94 conducted to study the impact of radioactivity on the communities of diatoms in tailing waters
95 (Ruggles, 1979 cited in Driver, 1994), brackish waters (Snoeijs and Notter, 1993), and in rivers
96 (Herlory, 2013) and mineral springs (Millan, 2019). High diatom species richness (>50) was
97 found in springs with significant levels of radioactivity by Millan et al. (2019). This study also
98 showed that high levels of ^{222}Rn activities in mineral springs are correlated with higher rates of
99 benthic diatom frustule deformations. All of these studies suggest that diatoms can resist high
100 radioactivity levels but did not provide any information on the effect of radioactivity on the
101 structure of diatom communities themselves.

102 In the present study, and in order to bring new insights at both population and community
103 levels, DNA metabarcoding was employed to assess the effect of enhanced natural radioactivity
104 of water and sediments on benthic diatom communities in several mineral springs of the Massif
105 Central. To this end, a 312 bp region of the chloroplast gene *rbcL* (encoding for the Ribulose
106 Bisphosphate Carboxylase Large) was used as a barcode for diatom species identification
107 (Vasselon, 2017), a convenient marker for such discriminations (Mann, 2010; Kermarrec, 2014;

108 Bailet, 2020). Following on from earlier published results of Millan et al. (2019), where species
109 richness was high even in radioactive mineral springs, our main working hypothesis was that
110 radioactivity is not a major factor that influences diatom genetic richness. Furthermore, because
111 microbial species have different levels of resistance to ionising radiation (Shuryak, 2019), our
112 second hypothesis was that radioactivity can influence diatom community structure because
113 ionising radiation can shape the microbial community. In this light, a series of studies were
114 performed in order to test these ideas empirically.

115 **2. Material and methods**

116 ***2.1. Study sites and sampling***

117 The sampling campaign took place in autumn from the 15th to the 30th October 2019, where
118 diatom samples were collected from a total of 16 mineral springs distributed in the Auvergne
119 region of the French Massif Central (Fig. 1). The French Massif Central (over 85,000 km²) is
120 influenced by oceanic, continental, and Mediterranean climates. It is composed of mountain
121 reliefs, plateaus, and volcanoes (quiescent), where the Hercynian platform appears at the
122 surface, surrounded by different sedimentary basins. Classically, the many faults in the massif
123 allow the circulation of water in the rocks and the emergence of this having travelled some
124 distance across the earth's crust. The study sites (mineral springs) present a diversity of
125 physical, chemical, and radiological characteristics including a three orders of magnitude
126 gradient of natural radioactivity.

127 ***2.2. Sampling of diatom communities***

128 Benthic diatom samples were collected in the 16 mineral springs (Fig. 1). Since it was earlier
129 demonstrated that the substrate has little influence on benthic diatom composition in mineral
130 springs (Baker, 2022), the biofilm was recovered and considered to be the dominant substrate,
131 representative of each of the springs sampled. In some springs, biofilm was collected by

132 scrubbing the upper surface of rocks over which water flowed with a clean toothbrush. In others,
133 biofilm was sampled by scraping the first millimetre or so of sediments from the spring basin.
134 For each sample, the suspension in the was fixed with using $\geq 70\%$ ethanol as required by
135 European protocols for diatom metabarcoding procedures (CEN, 2018).

136

137 **2.3. *Physical and chemical analyses***

138 At the 16 spring sampling sites, water temperature ($^{\circ}\text{C}$), conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$) and pH (pH
139 unit – Sorenson scale) were measured with a multi-parameter WTW probe FC 340i (VWR
140 International, Radnor, PA, USA) by submerging the probe in the water. The percentage of
141 dissolved oxygen (DO) was obtained with an Ysi ProODO oxygen probe (Yellow Springs
142 Instruments, OH, USA). For chemical analyses, 200 mL of water was initially filtered with
143 Whatman GF/C filters. For cation and anion analysis, a Thermo Scientific Dionex ICS1100 and
144 a Thermo Scientific Dionex Aquion systems were used, respectively, to measure concentrations
145 of the following ions (expressed as $\text{mg}\cdot\text{L}^{-1}$): lithium (Li^+), sodium (Na^+), ammonium (NH_4^+),
146 potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), fluoride (F^-), chloride (Cl^-), nitrite (NO_2^-),
147 nitrate (NO_3^-) and phosphate (PO_4^{3-}).

148 **2.4. *Radiological and trace elements characterization***

149 The activity of dissolved ^{222}Rn in water samples collected using 1 L Marinelli beakers was
150 measured on a well-shaped Germanium detector (GCW3523, Canberra, 35% relative
151 efficiency) operated at Clermont Physics Laboratory (University Clermont Auvergne, Aubière,
152 France) upon returning from the field. For other radiological analyses, water, and sediment
153 samples were collected in clean vials from the water-column either near the emergence or at its
154 outlet. Samples were transported to the laboratory under cold conditions in refrigerated boxes
155 protected from light and heat.

156

157 Analyses were conducted at Subatech (Nantes, France) and ICN (Nice, France) laboratories.
158 After PTFE filtration (0.45 μ m) and acidification of water samples (HNO₃ or HCl for Po
159 analysis), uranium was analysed by ICP-QMS (Xseries 2, Thermo Electron). Ultra-trace
160 concentrations of ²²⁶Ra were measured without chemical purification by HR-ICP-MS in low-
161 resolution mode (Element XR, Thermo Scientific) with an APEX-Q high sensitivity desolvating
162 sample solution introduction system. In order to remove major polyatomic interferences from
163 the spectrum of ²²⁶Ra in low-resolution mode thereby potentially leading to inaccurate
164 assessment of concentrations of this the commonest isotope of this highly radioactive element,
165 preliminary water sample solution analyses were also performed in medium resolution mode
166 where major interferences can be resolved. The observed consistency of ²²⁶Ra activity measured
167 in both low and middle resolution modes showed no significant evidence of the production of
168 major interferences (Verlinde, 2019). Preference for use of the low-resolution mode was
169 characterised by a high sensitivity (4.7 \times 10⁵ CPS/Bq. mL⁻¹) and a low instrumental detection
170 limit (0.8 mBq.L⁻¹) for ²²⁶Ra. Pb and Bi-based polyatomic interferences unresolved in low and
171 medium resolution modes were not considered in the present study due to low signal intensities
172 measured for Pb (< 60,000 CPS) and Bi (< 1 000 CPS). Lastly, ²¹⁰Po was measured after
173 spontaneous deposition onto a silver disc, by alpha spectrometry using dual alpha spectrometers
174 EG&G Ortec 576A equipped with boron-implanted silicon detectors (Le, 2019).

175 Sediment samples were sieved to 2mm and dried at 105°C. ²²⁶Ra and ²²⁸Ac were analysed
176 using a γ -Spectrometer with coaxial hyperpure Ge detector (GX4519, Mirion, >45% relative
177 efficiency at 1.33 MeV between 10 keV to 10 MeV) according to NF EN ISO 18589-3.
178 Measurements were conducted at least four weeks after the sealing of the sample container to
179 ensure the secular equilibrium between ²²⁶Ra, ²²²Rn, ²¹⁴Pb and ²¹⁴Bi. The data were then
180 processed via LabSOCS™ Calibration Software. ²³⁸U, ²³²Th and trace elements were measured

181 by ICP-QMS (Xseries 2, Thermo Electron) after microwave digestion (Ethos Easy Milestone)
182 with concentrated HNO₃, centrifugation at 2650g during 10 min, then PTFE filtration (0.45µm).

183 ***2.5. DNA extraction and PCR amplification for amplicons library preparation***

184 For each mineral spring, two sub-samples (duplicates) of 2 mL of biofilm were taken for
185 DNA extraction. After homogenization by manually shaking of the raw material, samples were
186 centrifuged at 13 000 rpm for 30 min. at 4°C to remove the supernatant (ethanol). Total genomic
187 DNA was extracted from the pellet with the commercial DNA extraction kit Nucleospin for
188 Soil (Macherey-Nagel, Düren, Germany). DNA quality of the samples was assessed by
189 spectrophotometry using the NanoDrop ND-1000 (Nanodrop Technologies, Wilmington,
190 Delaware). From each extraction duplicate, PCR amplifications targeting a 312 bp fragment of
191 the marker gene *rbcL* were done on two groups of triplicates. Amplification was performed
192 using the primers as described in Vasselon et al. (2017). Each PCR product group was pooled
193 to provide a PCR duplicate per extraction replicate. In summary, four replicates per mineral
194 spring were obtained. Library pools were sequenced on four Illumina MiSeq runs (2x250 bp
195 paired-end V3) by GenoToul GeT-PlaGe platform (INRAE Genomics, Toulouse, France).

196 ***2.6. Analysis of diatom communities***

197 The sequencing platform performed the demultiplexing and provided two fastq files per
198 mineral spring, one corresponding to forward reads (R1) and reverse reads (R2), respectively.
199 The R package DADA2 was used for the amplicon sequence variant (ASV) from the
200 demultiplexed Miseq following the method described by Callahan et al. (2016). An average of
201 132,420 sequences per sample were obtained before using DADA2 to filter the sequences.
202 Primers were removed from the R1 and R2 reads using CutAdap (Martin, 2011). Based on their
203 quality profile, R1 and R2 reads were truncated to 200 and 170 bp, respectively. Reads with
204 ambiguities and errors (maxEE) higher than 2 were eliminated. Then, chimeric sequences were

205 removed from the samples, resulting in an average of 120,364 sequences per sample. Amplicon
206 sequence variants (ASV) detected were assigned to taxa using version 10 of the curated
207 reference database for diatoms Diat.barcode (Rimet, 2021) available at
208 <https://doi.org/10.15454/D7FO2T>. ASV's not classified as Bacillariophyta were removed from
209 the analysis. A total of 565 ASVs was detected and assigned to the phylum of Bacillariophyta.
210 Several species and genera were not assigned taxonomically to any known sequence, as is
211 frequently the case in metabarcoding analyses (e.g. Rivera et al. 2020). Thus, an internal
212 database of GEOLAB (that will be added to Diat.barcode) allowed the identification of some
213 of the most abundant species belonging to the genus *Navicula* in springs, such as *N.*
214 *sanctamargaritae* Beauger, using light microscopy.

215 The diversity data of all samples were exploited using the 'Phyloseq' package (McMurdie,
216 2013) in R version 4.1.2. The files obtained from the DADA2 data processing: ASVs table,
217 taxonomy table and a table with the corresponding spring for each replicate were implemented
218 in R to create a phyloseq object and facilitate data manipulation. Sample α -diversity indices
219 (ASV richness and Shannon–Wiener's (Hill, 1973) diversity index) were calculated for each
220 replicate of the biological samples using the function "estimate_richness" (Fig. 2).

221 Replicates in metabarcoding allow better extrapolation of diatom diversity in mineral
222 springs. To reduce the number of files to be analysed, replicates of the same spring were merged
223 using the function "merge_sample." This function allows all ASVs present in the different
224 replicates to be combined as effectively representative of one sample per spring. Relative
225 abundances of species were calculated in each sample, and only species with an abundance >>
226 1% and representative of all the studied springs, were retained. The plot of the species
227 composition for all samples was built with the ggplot2 package (Fig. 3). The ecology of the
228 diatom species was determined using several diatom atlases, research papers and books (Cleve-
229 Euler, 1953; Carter and Bailey-Watts, 1981; Hartley et al, 1986; Krammer et al, 1997a, 1997b,

230 2000a, 2000b, 2004; Krammer, 2000, 2002; Potatova and Hamilton, 2007; Lange-Bertalot
231 1979, 2001; Lange-Bertalot et al, 2011, 2017; Bey and Ector, 2013a, 2013b; Beauger et al,
232 2015; Levkov et al, 2016; Li et al, 2018).

233 ***2.7. Statistical analysis***

234 Prior to performing statistical analyses, radiological measures were modified in order to obtain
235 the contribution of the spring. The limits of detection (LD) are very low and below the
236 environmental background noise. For the statistical study, samples below the LD were set to
237 "0". LD values were subtracted from all results.

238 Different statistical analyses were performed to test the effect of physical, chemical and
239 radiological variables on the biotic data. As abiotic data (table S1, S2) did not follow a normal
240 distribution, they were standardised using z-score normalisation $((x-\text{mean})/\text{standard deviation})$.
241 This transformation also allows comparison of variables that initially had different units. Forty-
242 nine (49) abiotic variables were available from measures in water and sediments of the mineral
243 springs monitored (Table S1, S2). To increase statistical robustness, collinearity tests were
244 performed in parallel with principal component analyses (PCA), and thus the final dataset was
245 reduced to 16 variables. These analyses were performed on the concentrations of major ions,
246 trace elements and radionuclides. Variables with a cosine of less than 0.4 on both axes of the
247 PCA were not retained. When two variables had a correlation coefficient $\gg 0.9$, the variable
248 with the higher cosine was retained as presented in Table 1. Thus, in the springs, some
249 radiologic parameters were more important and showed more variation in the spring water or
250 the sediment. Dissolved oxygen, conductivity, pH, and water temperature were added to the
251 dataset due to their ecological importance for diatoms (Patrick, 1977). A final PCA was
252 performed on the main environmental parameters selected in each spring.

253 The correlation between ASV richness and physico-chemical and radiological variables
254 was tested by calculating Pearson correlation coefficient with XLSTAT 2020 version 5.1, to
255 determine if these elements measured in our study influenced the number of diatom species in
256 mineral springs (Table 2).

257 In order to extract further information from our analyses, the different diatom
258 communities of mineral springs were compared. A matrix of dissimilarities based on Jaccard
259 dissimilarity index (Jaccard 1901) was generated with the function `vegdist` of the R package
260 “Vegan” (Oksanen et al., 2013, version 2.5–7). This index only takes into account the
261 absence/presence of species, so a second matrix with the abundance-based Jaccard dissimilarity
262 index “`ab.Jaccard`” (or `chao.jaccard`) described in Chao et al. (2006) was also generated. The
263 function “`dist.ldc`” of the package “Adespatial” version 0.3–14 (Dray et al. 2012) was used to
264 generate a dissimilarity matrix with this second index. The individual influence of five
265 environmental and radiological factors such as “Geographical location”, “ ^{238}U ”, “ ^{222}Ra ”,
266 “ ^{210}Po ”, “ ^{228}Ac ” activities level on diatom community diversity in springs were determined
267 (Table S3) with a non-parametric MANOVA analysis [i.e. ADONIS (Permutational
268 MANOVA)] based on Jaccard and “`ab.Jaccard`” dissimilarity indices using the function `Adonis`
269 of the `vegan` R package. The “geographical location” represents not only springs that belong to
270 the same geographical area but also to similar hydrogeological contexts linked to the fault
271 system and the geological layers associated. The radioactivity classes for four radionuclide
272 variables (i.e. ^{222}Rn , ^{238}U , ^{226}Ra , ^{210}Po level, ^{228}Ac) were determined from the orders of
273 magnitude observed for these variables in the sediments of the sampled springs (Table 1). For
274 ^{238}U and ^{226}Ra activities, three classes were determined by: Low: activity $< 0.01 \text{ Bq.L}^{-1}$;
275 Medium: $0.01 \text{ Bq.L}^{-1} \leq \text{activity} \leq 0.1 \text{ Bq.L}^{-1}$; High: activity $> 0.1 \text{ Bq.L}^{-1}$ and Low: activity $<$
276 0.1 Bq.g^{-1} ; Medium: $0.1 \text{ Bq.g}^{-1} \leq \text{activity} \leq 1 \text{ Bq.g}^{-1}$; High: activity $> 1 \text{ Bq.g}^{-1}$ respectively.
277 Similarly, ^{210}Po activity levels were Low: activity $< 0.00001 \text{ Bq.L}^{-1}$; Medium: $0.00001 \text{ Bq.L}^{-1}$

278 $\leq \text{activity} \leq 0.0001 \text{ Bq.L}^{-1}$; High: activity $> 0.0001 \text{ Bq.L}^{-1}$. For ^{228}Ac activity levels, the classes
279 were: Low: activity $< 1 \text{ Bq.g}^{-1}$; Medium: $1 \text{ Bq.g}^{-1} \leq \text{activity} \leq 10 \text{ Bq.g}^{-1}$; High: activity > 10
280 Bq.g^{-1} .

281

282 Further correlations between the 14 most abundant species and physico-chemical and
283 radiological variables were computed with XLSTAT 2020 version 5.1. The difference of ASV
284 structure between radioactivity classes (Table S3) was estimated using the Multi-Response
285 Permutation Procedure (MRPP) (Zimmerman, 1985, Mielke, 1991). MRPP is non-parametric,
286 multivariate approach that tests the null hypothesis that two or more predefined groups are not
287 different in species composition (here, predefined groups are geographical location and ^{238}U ,
288 ^{226}Ra , ^{210}Po , ^{228}Ac activities). The computed MRPP statistic delta (δ) represents the overall
289 weighted mean of within-group means of pairwise dissimilarities among plots. In addition, the
290 chance-corrected within-group agreement A statistic was computed (A = 0 means that in-group
291 and out-group heterogeneities are equal; A = 1 means that all members of each group are
292 identical and different from the members of other groups). This analysis was complemented
293 with an Indicator Species Analysis (ISA) (Dufrêne and Legendre's, 1997; McGeoch and
294 Chown, 1998) again using the vegan R package.

295 **3. Results**

296 *3.1. Environmental variables in mineral springs*

297 The physical, chemical, and radiological characteristics of the studied mineral spring sites are
298 reported in Table 1. Conductivity of the mineral springs varied between 3090 and 9680 $\mu\text{S.cm}^{-1}$
299 (mean of $5682.56 \pm 1693.30 \mu\text{S.cm}^{-1}$). pH did not display significant variations among the
300 mineral springs (mean \pm SD: 6.9 ± 0.3). The mean water temperature of the studied mineral
301 springs was 16.6 ± 3.6 °C. Mineral springs were not well oxygenated with a mean value of 22.3

302 $\pm 3.6\%$. Concentrations of Na^+ and HCO_3^- are high in mineral springs with an average of $939 \pm$
303 361 mg.L^{-1} and $2318 \pm 516 \text{ mg.L}^{-1}$ respectively. In the water, ^{238}U activity varied between
304 detection limit (2.5 mBq.L^{-1}) and 0.193 Bq.L^{-1} and ^{210}Po varied between 9 and $392 \mu\text{Bq.L}^{-1}$. In
305 the sediments, ^{226}Ra activity variation extended on almost two orders of magnitude, from 0.56
306 to 52.1 Bq.g^{-1} . Lastly, , considering all the springs, ^{228}Ac varied between detection limit (45
307 mBq.L^{-1}) and 28.8 Bq.g^{-1} .

308 Analysis of the data sets using PCA (Fig. 2) shows the distribution of springs based on
309 their physical, chemical and radiological parameters. Axis 1 and 2 explained, respectively,
310 41.53% and 24.34% of the data variability. Overall, PCA allows identification of four groups.
311 Thus, in the top quadrant, the springs for which the ^{226}Ra activity is significant, include
312 Daguillon, Graviers, Ours, Tennis and Tête de lion. Also, Chemin, Graviers, Ours, Salut,
313 Saulcée, Tennis and Tête de lion are represented by higher ^{228}Ac activity. Bard, Saladis,
314 Giraudon and Tennis springs show higher Ca^{2+} , HCO_3^- and K^+ concentrations. In the lower left
315 quadrant, Estreys, Sail, Salins, Saulcée and Trois sauts show high ^{210}Po , ^{238}U activities and
316 Mn^{2+} and PO_4^{3-} concentrations. In the lower right quadrant, Giraudon, Rocs bleus, Saladis are
317 associated with higher temperature, conductivity, pH and DO and also with high Br^- and Na^+
318 concentrations.

319 *3.2. Influence of physical, chemical and radiological factors on ASV richness*

320
321 A total of 565 ASV were detected. Figure 3 shows the number of ASVs observed and Shannon's
322 diversity index obtained for each mineral spring using the four technical replicates. The number
323 of ASVs ranged from 6 and 74 with a mean 29 ± 17 . The mineral spring with the most of ASVs
324 was "Salut" (i.e. 74 ± 7) and the lowest "Salins" (i.e. 8 ± 1). In terms of Shannon's diversity
325 index, the richest spring was "Chemin" (i.e. 2.38 ± 0.04), the poorest "Tennis" (i.e. 0.18 ± 0.09).

326 There was no significant correlation between ASV richness and physico-chemical or
327 radiological (^{238}U , ^{226}Ra , ^{210}Po , ^{228}Ac activities) parameters.

328 *3.3. Influence of environmental and radiological factors on diatom communities' structure*

329 The contribution of several environmental factors, including radiological parameters, to the
330 variability of diatom communities in mineral springs was determined using an Adonis test
331 (Table 3). These contributions were calculated by taking into account the ASVs shared between
332 samples (Jaccard) and the abundance of these ASVs (ab.Jaccard). The geographical location of
333 the mineral springs explains, respectively, 47.0 % (Jaccard) and 53.5% (ab.Jaccard) of the ASV
334 variability. For the radiological parameters, ^{238}U activities in the spring water explains 17.2 %
335 (Jaccard) and 38.6 % * (ab.Jaccard) of the variability while its descendant ^{226}Ra activity in the
336 spring sediments explains 10.7 % (Jaccard) and 7.7 % (ab.Jaccard). ^{210}Po activities in the
337 springs' water contributes to 5.6 % (Jaccard) and 0 % (ab.Jaccard) of the variability while ^{228}Ac
338 activities in the springs sediments explains 13.3 % (Jaccard) and 3.5 % (ab.Jaccard) of this
339 variability.

340 *3.4. Taxonomic composition of diatom communities in mineral springs*

341 Sixty percent of the ASVs were assigned to a diatom genus, forty-two percent to a species.
342 Fifty-two genera were determined of which only 22 were associated to ASVs with read
343 abundance > 10 % in all samples. The dominant genera were *Achnantheidium*, *Amphora*,
344 *Anomoeoneis*, *Caloneis*, *Craticula*, *Cyclotella*, *Diatoma*, *Diploneis*, *Epithemia*, *Fallacia*,
345 *Fistulifera*, *Fragilaria*, *Gedaniella*, *Gomphonema*, *Halamphora*, *Mayamaea*, *Navicula*,
346 *Nitzschia*, *Pinnularia*, *Planothidium*, *Sellaphora*, *Surirella*. The most dominant ASV (relative
347 abundance above 1% in at least on sample) were assigned to 32 diatom species (Figure 4): 69%
348 of the diatom species are freshwater species, 17% found in both freshwater and marine
349 ecosystems, 7% in brackish water, and 7% in marine habitats. Among the 20 dominant ASVs,

350 some could not be assigned to a species and belong to the genera *Gomphonema*, *Navicula*,
351 *Pinnularia* and *Surirella*. The other dominant assigned species are *Navicula sanctamargaritae*,
352 *Gedaniella* sp., *Planothidium frequentissimum*, *Navicula veneta*, *Diploneis vacillans*, *Amphora*
353 *copulata*, *Pinnularia brebissonii*, *Halamphora coffeaeformis*, *Gomphonema saprophilum*, and
354 *Nitzschia vitrea*. Different ASVs were assigned to the same diatom species suggesting the
355 presence of genetic variants.

356 Pearson correlations between the abundance of the 14 dominant ASVs and the physical,
357 chemical and radiological parameters were determined (Table S4). All significant correlations
358 were positive. Na⁺ was correlated with *Pinnularia* sp2 (R²=0.60), which also correlated with
359 several parameters as PO₄³⁻ (R²=0.60), conductivity (R²=0.52), pH (R²=0.51), DO (R²=0.54),
360 temperature (R²=0.71). *Navicula veneta* correlated with PO₄³⁻ (R=0.68) and temperature
361 (R²=0.76). *Pinnularia brebissonii* with pH (R²=0.65) and DO (R²=0.83). The genetic variant A
362 of *Planothidium frequentissimum* correlated with ²³⁸U activity in water (R²=0.91) and ²¹⁰Po
363 activity in water (R²=0.91), but no correlation was found for the genetic variant B. Additional
364 analyses (MRPP; Table 4) showed that there is a significant difference in ASV composition
365 between samples with different location (with Jaccard matrix: A=0.146, p=0.002) and different
366 ²³⁸U activity levels (in water) (with ab.Jaccard matrix: A=0.230, p=0.004). No significant
367 differences were found between ASV composition for the different levels of the other
368 radionuclides. The Indicator Species Analysis revealed that the ASV of the genetic variant A
369 of *Planothidium frequentissimum* was associated with high level of ²³⁸U (in water) (R²=0.998,
370 p=0.01).

371 **4. Discussion**

372 This study provides the first analysis of diatom communities living in mineral springs using
373 DNA metabarcoding to assess the influence of enhanced natural radioactivity on these
374 assemblages. These extreme aquatic environments in effect comprise a field laboratory in which

375 to assess the contribution of natural radioactivity through radionuclides' activity on diatom
376 community structure. From this, two hypotheses were posited: (1) that radioactivity is not a
377 factor that particularly influences diatom genetic richness; and (2), that radioactivity is a factor
378 that can influence diatom community structure.

379 *4.1. Natural radioactivity did not impact ASV richness*

380 A first parameter to describe the α -diversity of communities with metabarcoding is ASV
381 richness. Generally, diatom ASV richness was high in the springs monitored and varied greatly
382 between mineral springs with a factor of about 11. No significant correlations were observed
383 between ASV richness and the various radiological parameters, viz. ^{238}U , ^{226}Ra , ^{210}Po , ^{228}Ac
384 activity. These results validate the first hypothesis: radioactivity does not influence diatom
385 community genetic ASV richness. In the literature and to our knowledge, no other study besides
386 the present one has yet been conducted on the effect of radioactivity on ASV richness of
387 microbial communities. Even so, a negative impact of radioactivity on the species richness of
388 macroorganisms in terrestrial ecosystems are a reduction of species richness, for instance forest
389 birds in an area with high radiation doses around Chernobyl (Moller, 2007; Mousseau 2016).
390 For microorganisms, Shuryak et al. (2018) suggest that diatom richness in some radioactive
391 waterbodies in Russia can be greatly reduced by high radiation levels: in this particular study,
392 a negative correlation of -0.81 was measured between diatoms species richness and the level of
393 radioactive contamination. The result obtained in our study is significantly higher than that
394 observed in the monitored mineral springs because radioactive levels are elevated by a factor
395 of eight in the radioactive reservoirs in Russia. In another study, comparison of bacterial
396 communities in geographically-separated soils affected by caesium releases showed that
397 species richness was lower in the most contaminated soils (Ihara, 2021). However, in aquatic
398 environments, different results are reported: for example, Herlory et al. (2013), revealed that
399 diatom species richness was not affected by the treated uranium mining effluents; Videvall et

400 al. (2022) that radioactivity had no effect on prokaryote richness in Chernobyl wetlands, whilst
401 Murphy et al. (2011) also showed that radioactivity had no effect on macroinvertebrate
402 communities from lakes exposed to Chernobyl-derived ionising radiation. The latter results
403 were obtained in the context of anthropogenic contamination by the addition of radionuclides
404 to the environment, which implies stress on the surrounding organisms due to the introduction
405 of these elements. In the case of radioactive mineral springs, organisms grow in the presence
406 of radioactivity for long periods. Thus, we assume that organisms are adapted to radioactivity
407 in mineral springs where the radioactive level is higher.

408 *4.2. Uranium activity explained part of diatom community structure*

409 Geographical location was found to be the main factor explaining diatom community structure
410 in mineral springs considering ASV occurrence. This result is similar to those of Potapova and
411 Charles (2002), Virtanen and Soininen (2012), Soininen et al. (2016) and Baker et al. (2021)
412 that studied benthic diatom assemblages in rivers, streams and springs. ^{238}U activity was the
413 second parameter that explained the variability of the diatom communities, whereas the
414 variability of diatom community structure was more poorly explained by ^{226}Ra , ^{210}Po and ^{228}Ac
415 activities.

416 In the literature, study of the effects of uranium on communities of various macro- and
417 microorganisms is quite common in the context of anthropogenic contamination or laboratory
418 experiments. Several studies have demonstrated that uranium in water courses and static bodies
419 of water influences the taxonomic composition of microbial communities (Reardon, 2004;
420 Islam, 2011; Brzoska, 2016; Sutcliff, 2017; Suriya, 2017; Zeng, 2018; Chuiyun, 2021; Videvall,
421 2022). For example, Suriya et al. (2017) showed that increased concentration of uranium in
422 sediments of the Cauvery River in India favour the presence of several Proteobacteria and
423 Gammaproteobacteria species. Exposure of the bacterial community found in anaerobic
424 granular sludge to uranium leads to the modification of the composition of the community

425 (Zeng, 2018). However, there do not seem to be any studies on the effect of naturally occurring
426 uranium in the environment. This study is therefore a pioneer study on diatom community
427 structure in natural radioactive areas. Moreover, uranium contamination (from mining, nuclear
428 accidents, or the release of nuclear waste into the environment) is a relatively recent event, as
429 it is linked to the growing demand for uranium by the domestic and military industries since
430 the Second World War. In the context of mineral springs, microorganisms have been naturally
431 exposed to uranium in a continuous and prolonged manner, and uranium activity seems to have
432 a significant influence on the structure of the communities, especially considering the genetic
433 variants present.

434 This last view validates the second hypothesis: radioactivity is a factor that can influence
435 diatom community structure. Furthermore, bioaccumulation of ^{226}Ra by several organisms such
436 as plants, algae, bacteria, plankton and zooplankton is also documented to occur (Iyengar, 1990;
437 Zakeri, 2012; Carvalho, 2014; Heidari, 2017). ^{226}Ra accumulates in a similar way to calcium in
438 human and animal body tissues, by accumulating on the surface of bones, whilst also leading
439 to the cancers of various kinds (Gad, 2005; Gad, 2014). Diatoms can also absorb and use
440 calcium in their metabolic processes calcium and therefore it is also likely that ^{226}Ra is
441 accumulated by diatoms and thereby involved in their metabolism too. ^{210}Po bioaccumulation
442 is well documented in marine environments (Bennett, 1979; Carvalho, 2018; Uddin, 2018),
443 marine phytoplankton (Horowitz, 2020), and invertebrates from the Baltic sea (Skwarze and
444 Falkowski, 1988). All these radionuclides can be accumulated in cells of living organisms.
445 Brown et al. (2004) observed that ^{210}Po and ^{226}Ra are the main contributors of absorbed dose
446 rates when they measured the internal concentration of several radionuclides within marine and
447 freshwater organisms (e.g., phytoplankton, macrophytes). However, in our study, ^{226}Ra and
448 ^{210}Po are the two radionuclides that contribute the least to explanation of the community
449 structure of diatoms frequenting natural bodies of water.

450 4.3. Taxonomic composition of diatom communities

451 The main challenges and limitations of the metabarcoding method as here described are the
452 reference barcoding libraries themselves. Indeed, these reference libraries are often incomplete,
453 and difficult to produce and maintain (e.g. Weigand et al., 2019). Morphological discrimination
454 under a powerful microscope is still found to be necessary for species identification and can in
455 turn be complemented by metabarcoding. The lack of genus and/or species identification could
456 also be explained by the presence of new cryptic taxa due to the insularity of mineral springs.
457 Indeed, springs are biotopes that can be marked by insularity via physical or physico-chemical
458 barriers. Such barriers are characterised by high levels of physical stress related for example to
459 temperature water availability and radioactivity (Cantonati, 2012; Cartwright, 2019). Thus, they
460 present specific biotopes distinct from surrounding ecosystems and as such, may contain novel
461 species due to potential allopatric speciation events.

462 A large proportion of the taxa herein observed by us could not be identified down to
463 species level, but the general ecology of those that could be so identified demonstrated that
464 most of those colonizing springs are present in various types of environments (freshwater,
465 marine species and brackish water). Furthermore, their distributions are related to some
466 chemical similarities between mineral springs and marine or brackish environment such as high
467 conductivity, high ions contents among others. Also, some diatoms species have large
468 ecological range and inhabited environments with different ecological statue like freshwater,
469 springs and brackish water. Only *Navicula sanctamargaritae* is typical to mineral springs
470 (Beauger et al., 2020). Thus, the observations made in the mineral springs show that some
471 species are well distributed in the mineral springs, but their abundance varies according to the
472 local environmental conditions.

473 The correlations between the species associated to the 14 most abundant ASVs and
474 physical, chemical and radiological parameters of the springs monitored clearly revealed that

475 one genetic variant of *Planothidium frequentissimum* is particularly abundant in mineral springs
476 with high natural concentrations of the isotope ^{238}U . This result was confirmed with MRPP and
477 ISA, which revealed interestingly that this species is associated with high ^{238}U activity. Hence,
478 this finding means that there are clearly genetic variants of the same diatom species that do not
479 have the same distribution and ecological tolerances. This finding agrees with those of Pérez-
480 Burillo et al. (2021), working on the distribution and ecological preferences of four diatom
481 species, who concluded that ecological tolerance of some diatom species resulted from
482 overlapping preferences among genetic variants. These genetic variants individually showed
483 much more restricted preferences and distributions. In a previous study, a higher frequency of
484 deformations in the most radioactive springs had been observed for *P. frequentissimum* (Millan,
485 2019).

486 Mineral springs with high levels of radioactivity (i.e. ^{238}U activity) are habitats in which
487 environmental pressure is more important than springs with low levels of radioactivity. If so, it
488 questions the ability of this species to tolerate intracellular accumulation of radionuclides.
489 Indeed, bioaccumulation of radionuclides depends on several environmental factors including
490 external concentrations of these radionuclides in the water. In this context, Snoeijs and Notter
491 (1993), demonstrated that external concentrations of radionuclides and benthic diatom
492 concentrations are related: the higher the radionuclides concentrations, the higher the
493 intracellular concentrations in the diatoms. This thus strongly suggests that in environments
494 with high concentrations of radionuclides, diatom species are able to tolerate high radionuclide
495 concentrations. If this in turn is so, the genetic variant A of *P. frequentissimum* is a potentially
496 a good bio-indicator species for high natural uranium levels in spring water. Lastly, diatoms are
497 the main producers in aquatic environments and the possible high accumulation of
498 radionuclides within the cells implies – importantly – that diatoms are themselves potential
499 vectors of radionuclides in the high levels of the food webs.

500 **Conclusions**

501 Mineral springs are extreme environments that may have naturally high levels of radioactivity.
502 Such environments are good field ‘laboratories’ to observe and understand the adaptation of
503 organisms in the face of higher levels of natural and chronic background radioactivity. Our
504 present study, the first on diatom community structure in natural radioactive areas, reveals that
505 DNA metabarcoding proves that diatom species are very abundant in the mineral springs of the
506 Massif Central. Radioactivity in the springs has no apparent effect on the diatom ASV richness
507 in these mineral springs. Geographical location is the first factor explaining the structure of
508 diatom communities in the mineral springs, and ^{238}U the second factor. This radionuclide is
509 known to influence microbial community composition. Very interestingly, one of the genetic
510 variants of *Planothidium frequentissimum* was detected as a bio-indicator of high level of
511 uranium in natural environment (i.e., mineral springs). Lastly, our results show that some
512 diatom species are well distributed in the mineral springs, but their abundance varies according
513 to the environmental conditions found locally. Thus, some species are particularly abundant in
514 mineral springs with a high enhanced level of natural radioactivity. This exemplifies the fact
515 that diatoms are organisms that have a great capacity for tolerance to radionuclides.

516 **Acknowledgements**

517 We thank François Keck for his expertise and advices in terms of analysis of the metabarcoding
518 data, Hugh D. Loxdale for his English editing, and the two anonymous reviewers for their
519 valuable comments, which have greatly improved the manuscript.

520 **Funding sources:** This study was funded by a grant from the Centre National de la Recherche
521 Scientifique (NEEDS, project Acronym: DISCOVER)) including a CNRS doctoral grant for
522 L.A. BAKER. We acknowledge financial support from CNRS-INEE within the context of the
523 Zone Atelier Territoires Uranifères and the Clermont Auvergne Metropole.

524 **Conflicts of Interest:** The authors declare no conflict of interest.

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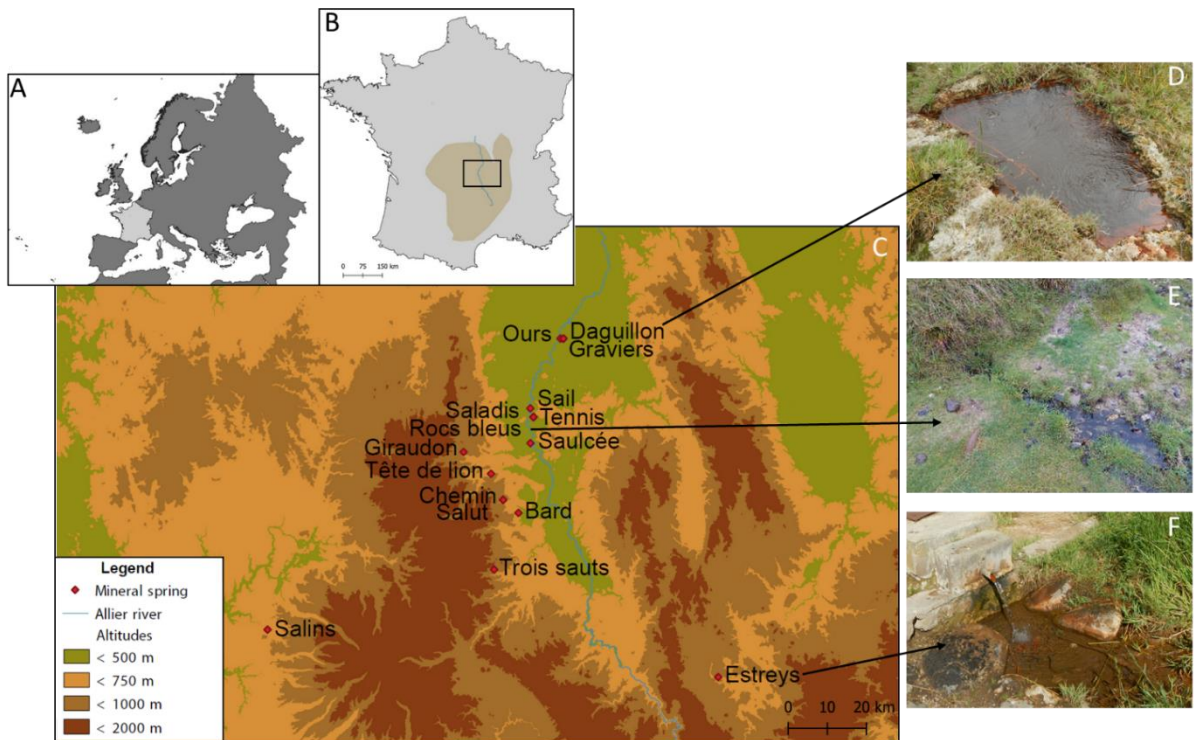
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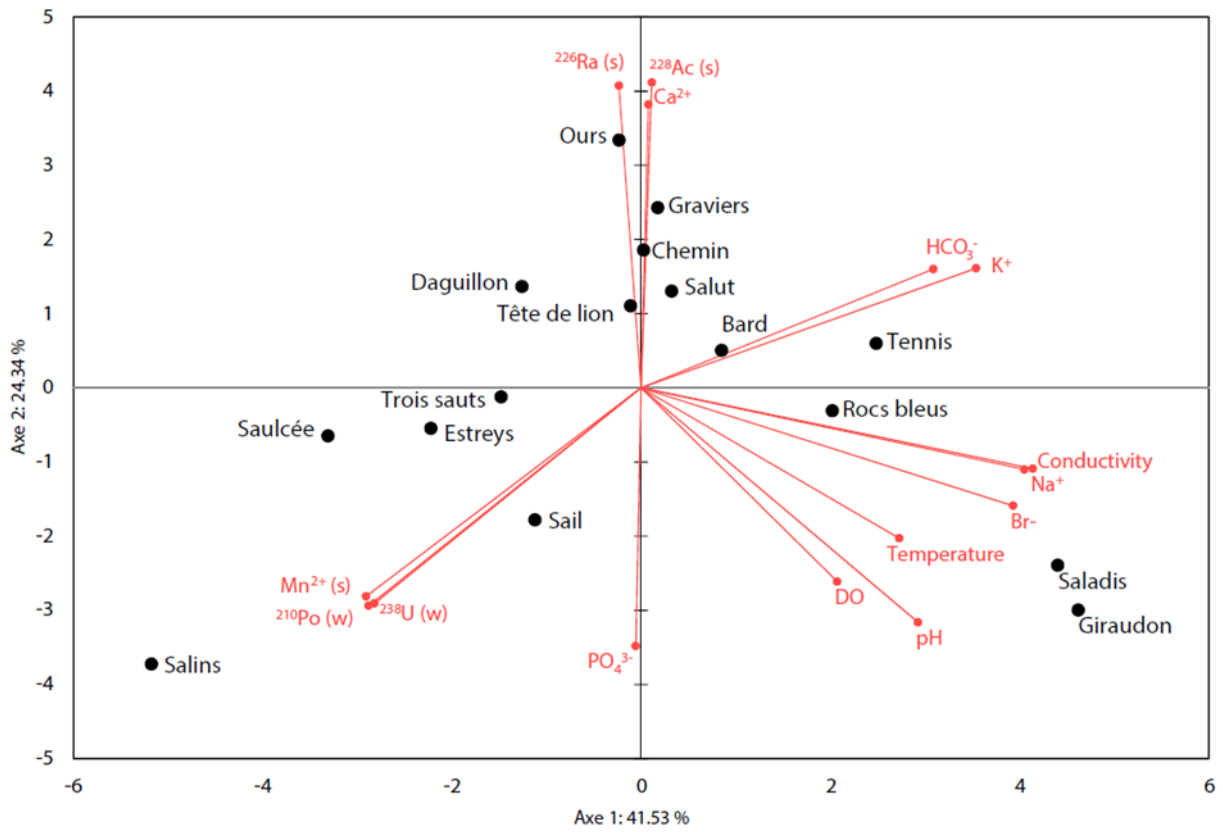
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984
 985 **Figure 1:** Location map of the study sites. (A) Map of Europe, (B) Map of the Massif Central in France, (C) Map
 986 of the 16 mineral springs monitored in the Massif Central; photographs of some mineral springs (D, E, F).
 987 monitored.

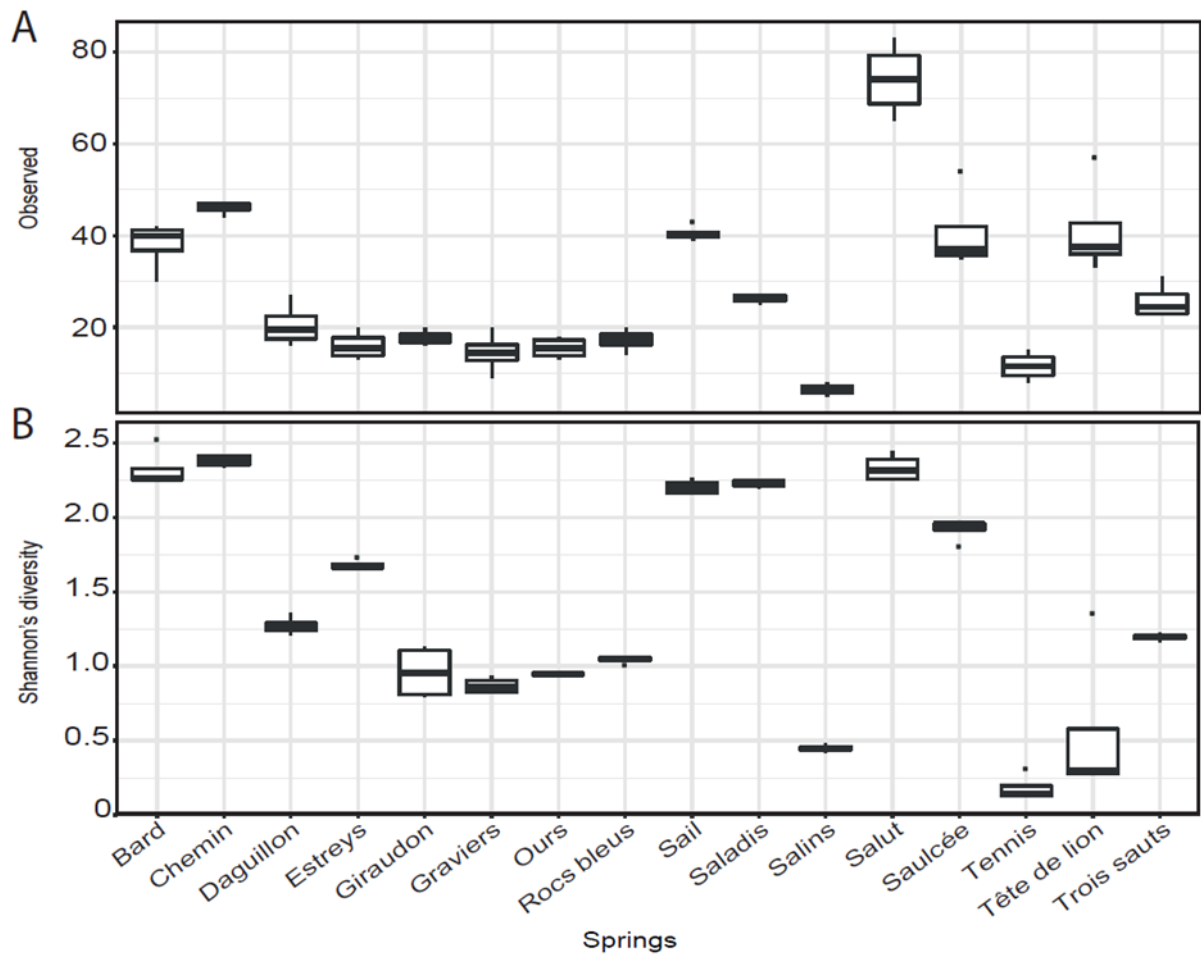
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990 **Figure 2:** Principal component analysis (PCA) shows the springs' distribution based on physical, chemical and
 991 radiological variables measured in water (w) or sediments (s).

992



993

994 **Figure 3:** Boxplot showing the mean of (A) ASV richness corresponding to benthic diatom species richness and

995 (B) Shannon's diversity for each technical replicate (N=4) of the mineral springs monitored in the Massif Central

996 (France).

997

Table 1: Physical, chemical and radiological variables measured at the mineral springs monitored in the Massif Central (France) after the selection of variables with stepwise followed by the subtraction of detection limits (DL). Cond: conductivity; DO: dissolved oxygen; Temp: Temperature. ^{238}U , ^{210}Po ^{226}Ra ; ^{228}Ac expressed radionuclides activity in spring water (w) or sediments (s).

Springs	Na ⁺ (mg.L ⁻¹)	K ⁺ (mg.L ⁻¹)	Ca ²⁺ (mg.L ⁻¹)	Br ⁻ (mg.L ⁻¹)	PO ₄ ³⁻ (mg.L ⁻¹)	HCO ₃ ⁻ (mg.L ⁻¹)	Cond (μS.cm ⁻¹)	pH	DO (%)	Temp (°C)	²³⁸ U (w) (Bq.L ⁻¹)	²²⁶ Ra (s) (Bq.g ⁻¹)	²¹⁰ Po (w) (μBq.L ⁻¹)	²²⁸ Ac (s) (Bq.g ⁻¹)	Mn ²⁺ (s) (mg.g ⁻¹)
Bard	1,028.88	185.88	233.76	1.88	0.12	3,012	6,380	6.81	3.1	14.7	0	2.2	0	0.6	0.33
Chemin	990.42	179.01	202.55	1.72	0.09	2,515	4,860	6.41	15.3	14.2	0.002	10.8	0	26.1	0.24
Daguillon	503.16	112.22	297.06	0.56	0.13	2,142	4,030	6.6	29	13.9	0	23.0	0	4.6	0.30
Estreys	504.61	45.10	128.21	1.65	0.11	1,185	3,480	6.38	16.4	16	0.012	1.9	17	0.0	0.40
Giraudon	1,787.50	189.50	151.28	4.91	0.70	2,814	9,201	7.51	54	32.1	0	4.2	0	5.9	0.24
Graviers	823.10	159.59	381.87	1.66	0.13	2,730	5,430	6.69	19.5	14.4	0	26.7	0	17.9	0.39
Ours	713.30	146.24	313.27	1.20	0.16	2,648	5,490	6.72	13.8	13.7	0	52.1	0	28.7	0.11
Rocs bleus	1,396.77	144.23	255.14	3.68	0.06	2,696	8,150	7	9.3	19.2	0	0.6	0	0.1	0.16
Sail	686.62	83.46	85.67	1.13	0.54	1,150	5,390	7.02	9.2	16.6	0.004	0.8	0	0.0	0.32
Saladis	1,813.91	197.52	112.55	4.09	0.05	2,928	9,680	7.82	88.6	16.1	0	1.2	0	0.1	0.17
Salins	291.27	53.52	204.16	0.23	0.53	1,948	3,090	6.83	20.1	13.3	0.193	0.8	382	0.4	9.32
Salut	1,041.30	189.46	179.92	1.67	0.09	2,740	5,850	6.46	11.7	12.1	0	9.5	0	12.8	0.10
Saulcée	464.48	67.75	173.14	0.95	0.05	1,412	3,420	6.66	26	14.4	0.082	19.3	158	11.7	5.35
Tennis	1,408.88	154.92	281.91	3.34	0.09	2,778	8,380	7.05	8.9	27.1	0	21.6	0	11.9	0.11
Tête de lion	768.30	147.04	239.54	1.62	0.08	2,454	4,200	6.99	18.9	14.4	0	15.9	0	12.9	0.41
Trois sauts	803.89	69.23	135.35	0.89	0.06	1,940	3,890	6.66	12.8	13.1	0.004	2.1	0	1.1	0.53

Table 2: Pearson correlation coefficient (R) between ASV richness of benthic diatom communities and the physical, chemical and radiological variables measured in studied mineral springs of the Massif Central (N=16). ASV: Amplicon sequence variant richness, Cond: Conductivity, DO: Dissolved oxygen, Temp: Temperature, ²³⁸U; ²¹⁰Po ²²⁶Ra; ²²⁸Ac expressed radionuclides activity in spring water (w) or sediments (s). Values in bold are significant p-value ($\alpha = 0.05$).

	ASV	Na ⁺	K ⁺	Ca ²⁺	Br ⁻	PO ₄ ³⁻	HCO ₃ ⁻	Cond	pH	DO	Temp	²³⁸ U (w)	²²⁶ Ra (s)	²¹⁰ Po (w)	²²⁸ Ac (s)	Mn ²⁺ (s)
ASV	1	-0.01	0.29	-0.32	-0.15	-0.23	0.01	-0.11	-0.26	-0.15	-0.36	-0.25	-0.15	-0.25	0.18	-0.21
Na ⁺	-0.01	1	0.75	-0.13	0.95	0.05	0.68	0.96	0.73	0.53	0.66	-0.49	-0.23	-0.48	-0.08	-0.50
K ⁺	0.29	0.75	1	0.27	0.63	-0.12	0.90	0.73	0.40	0.30	0.29	-0.54	0.13	-0.52	0.35	-0.55
Ca ²⁺	-0.32	-0.13	0.27	1	-0.12	-0.29	0.48	-0.03	-0.25	-0.32	-0.07	-0.10	0.70	-0.08	0.53	-0.09
Br ⁻	-0.15	0.95	0.63	-0.12	1	0.13	0.56	0.93	0.73	0.52	0.78	-0.42	-0.24	-0.41	-0.14	-0.43
PO ₄ ³⁻	-0.23	0.05	-0.12	-0.29	0.13	1	-0.17	0.11	0.35	0.15	0.47	0.35	-0.25	0.34	-0.24	0.30
HCO ₃ ⁻	0.01	0.68	0.90	0.48	0.56	-0.17	1	0.67	0.37	0.22	0.26	-0.35	0.19	-0.33	0.30	-0.36
Cond	-0.11	0.96	0.75	-0.03	0.93	0.11	0.67	1	0.77	0.48	0.68	-0.45	-0.13	-0.43	-0.11	-0.46
pH	-0.26	0.73	0.40	-0.25	0.73	0.35	0.37	0.77	1	0.73	0.56	-0.09	-0.24	-0.08	-0.34	-0.09
DO	-0.15	0.53	0.30	-0.32	0.52	0.15	0.22	0.48	0.73	1	0.26	-0.02	-0.16	-0.01	-0.19	-0.01
Temp	-0.36	0.66	0.29	-0.07	0.78	0.47	0.26	0.68	0.56	0.26	1	-0.20	-0.12	-0.20	-0.13	-0.21
²³⁸ U (w)	-0.25	-0.49	-0.54	-0.10	-0.42	0.35	-0.35	-0.45	-0.09	-0.02	-0.20	1	-0.17	1	-0.20	0.99
²¹⁰ Po (w)	-0.25	-0.48	-0.52	-0.08	-0.41	0.34	-0.33	-0.43	-0.08	-0.01	-0.20	1	-0.16	1	-0.19	0.99
²²⁶ Ra (s)	-0.15	-0.23	0.13	0.70	-0.24	-0.25	0.19	-0.13	-0.24	-0.16	-0.12	-0.17	1	-0.16	0.79	-0.14
²²⁸ Ac (s)	0.18	-0.08	0.35	0.53	-0.14	-0.24	0.30	-0.11	-0.34	-0.19	-0.13	-0.20	0.79	-0.19	1	-0.17
Mn ²⁺ (s)	-0.21	-0.50	-0.55	-0.09	-0.43	0.30	-0.36	-0.46	-0.09	-0.01	-0.21	0.99	-0.14	0.99	-0.17	1

Table 3: Non-parametric Multivariate Analysis of Variances (NPMANOVA or Adonis) results for unifactorial analysis of location, ^{238}U , ^{226}Ra , ^{210}Po and ^{228}Ac activity levels in spring water (w) or sediments (s), made with Jaccard (J) and ab.Jaccard (ab.J) distances performed on ASV matrix with relative abundances. The matrix contains only ASV with a relative abundance of more than 2% in at least one sample of the mineral springs monitored in the Massif Central (N=16). Significant p-value is indicated with (*).

Factors	R² (J)	R² (ab.J)
Location	47.01 %	53.53 %
^{238}U activity level (w)	17.18 %	38.59 % *
^{226}Ra activity level (s)	10.69 %	7.74 %
^{210}Po activity level (w)	5.64 %	0
^{228}Ac activity level (s)	13.34 %	3.45 %

Table 4: Multiple Response Permutation Procedure (MRPP) results for analysis of differences of community structure between sample group of location, ²³⁸U, ²²⁶Ra, ²¹⁰Po and ²²⁸Ac activity levels in spring water (w) or sediments (s) made with Jaccard (J) and ab.Jaccard (ab.J) distances performed on ASV matrix with relative abundances. The matrix contains only ASV with a relative abundance of more than 2% in at least one sample of the mineral springs monitored in the Massif Central (N=16). MRPP is based on 999 permutations. ASV: Amplicon sequence variant richness, Cond: Conductivity, DO: Dissolved oxygen, Temp: Temperature.

Distance index	Geographic location	²³⁸U Activity	²²⁶Ra Activity	²¹⁰Po Activity	²²⁸Ac Activity	
(J)	Chance-corrected within-group A	0.146	0.026	-0.049	-0.010	0.005
	Observed δ	0.674	0.768	0.455	0.797	0.785
	Expected δ	0.789	0.789	0.434	0.789	0.789
	Significance of δ	p=0.002	p>0.05	p>0.05	p>0.05	p>0.05
(ab.J)	Chance-corrected within-group A	0.368	0.230	-0.011	-0.040	0.005
	Observed δ	0.274	0.334	0.797	0.451	0.785
	Expected δ	0.434	0.434	0.789	0.434	0.789
	Significance of δ	p=0.11	p=0.004	p>0.05	p>0.05	p>0.05

Supplementary Table S1: Physical and chemical variables measured in the mineral springs monitored in the Massif Central (France). N=16

Springs	Na⁺ (mg.L⁻¹)	K⁺ (mg.L⁻¹)	Ca²⁺ (mg.L⁻¹)	Br⁻ (mg.L⁻¹)	PO₄³⁻ (mg.L⁻¹)	HCO₃⁻ (mg.L⁻¹)	Cond (μS.cm⁻¹)	pH	DO (%)	Temp (°C)
Bard	1028.88	185.88	233.76	1.88	0.12	3012	6380	6.81	3.1	14.7
Chemin	990.42	179.01	202.55	1.72	0.09	2515	4860	6.41	15.3	14.2
Daguillon	503.16	112.22	297.06	0.56	0.13	2142	4030	6.6	29	13.9
Estreys	504.61	45.10	128.21	1.65	0.11	1185	3480	6.38	16.4	16
Giraudon	1787.50	189.50	151.28	4.91	0.70	2814	9201	7.51	54	32.1
Graviers	823.10	159.59	381.87	1.66	0.13	2730	5430	6.69	19.5	14.4
Ours	713.30	146.24	313.27	1.20	0.16	2648	5490	6.72	13.8	13.7
Rocs bleus	1396.77	144.23	255.14	3.68	0.06	2696	8150	7	9.3	19.2
Sail	686.62	83.46	85.67	1.13	0.54	1150	5390	7.02	9.2	16.6
Saladis	1813.91	197.52	112.55	4.09	0.05	2928	9680	7.82	88.6	16.1
Salins	291.27	53.52	204.16	0.23	0.53	1948	3090	6.83	20.1	13.3
Salut	1041.30	189.46	179.92	1.67	0.09	2740	5850	6.460	11.7	12.1
Saulcée	464.48	67.75	173.14	0.95	0.05	1412	3420	6.66	26	14.4
Tennis	1408.88	154.92	281.91	3.34	0.09	2778	8380	7.05	8.9	27.1
Tête de lion	768.30	147.04	239.54	1.62	0.08	2454	4200	6.99	18.9	14.4
Trois sauts	803.89	69.23	135.35	0.89	0.06	1940	3890	6.66	12.8	13.1

Supplementary Table 2: Raw data of the radiological parameters and trace elements measured in the mineral springs monitored in the Massif Central (France). NA in the column “ ^{226}Ra (Bq.L $^{-1}$)” specified that this variable could not be measured for the sample.

Springs	Water		Sediments		
	^{238}U (Bq.L $^{-1}$)	^{226}Ra (Bq.L $^{-1}$)	^{210}Po ($\mu\text{Bq.L}^{-1}$)	^{228}Ac (Bq.g $^{-1}$)	Mn^{2+} (mg.g $^{-1}$)
Bard		0.922	<10	0.62	0.333
Chemin	0.004428	1.15	<10	26.11	0.237
Daguillon	<2.5E-3	3.221	<10	4.60	0.301
Estreys	<2.5E-3	1.204	27	0	0.400
Giraudon	<2.5E-3	NA	<10	5.91	0.235
Graviers	0.014514	2.331	<10	17.92	0.391
Ours	<2.5E-3	2.621	<10	28.72	0.105
Rocs bleus	<2.5E-3	NA	<10	0.05	0.158
Sail	0.006765	0.472	<10	0	0.321
Saladis	<2.5E-3	2.013	<10	0.07	0.170
Salins	0.195693	0.736	392	0.41	9.323
Salut	<2.5E-3	1.548	<10	12.78	0.097
Saulcée	0.084255	1.438	168	11.65	5.351
Tennis	0.002706	3.338	<10	11.88	0.112
Tête de lion	0.002583	NA	<10	12.87	0.413
Trois sauts	0.00615	<1.4E-1	<10	1.09	0.530

Supplementary Table 3: Qualitative variables of the mineral springs monitored in the Massif Central indicating geographical location, and activity levels of ^{238}U , ^{210}Po , ^{226}Ra and ^{228}Ac in water (w) or in sediment (s).

Springs	Geographical location	^{238}U level (w)	^{226}Ra level (s)	^{210}Po level (w)	^{228}Ac level (s)
Bard	Boudes	low	medium	low	low
Chemin	Ternant les eaux	medium	high	low	high
Daguillon	Joze	low	high	low	medium
Estreys	Polignac	low	medium	medium	low
Giraudon	Saint-Nectaire	low	medium	low	medium
Graviers	Joze	low	high	low	high
Ours	Joze	low	high	low	high
Rocs bleus	Martes-de-Vevre	low	low	low	low
Sail	Mirefleurs	low	low	low	low
Saladis	Martes-de-Vevre	low	medium	low	low
Salins	Apcher	high	low	high	low
Salut	Ternant les eaux	low	medium	low	high
Saulcée	Coudes	medium	high	high	high
Tennis	Sainte-Marguerite	low	high	low	high
Tête de lion	Saint-Floret	low	high	low	high
Trois sauts	Leyvaux	low	medium	low	medium

Supplementary table 4: Pearson correlations between the abundance of the 14 dominant species and the radiological parameters ^{238}U , ^{226}Ra , ^{210}Po , ^{228}Ac , activities and trace element concentration (Mn^{2+}) in the springs monitored in the Massif Central (N=16). Significant p-value are in bold ($p < 0.05$). Species are classified with decreasing their gradient of relative abundances in the mineral. Cond: Conductivity, DO: Dissolved oxygen, Temp: Temperature.

	Na^+	K^+	Ca^{2+}	Br^-	PO_4^{3-}	HCO_3^-	Cond	pH	DO	Temp	^{238}U (w)	^{210}Po (w)	^{226}Ra (s)	^{228}Ac (s)	Mn^{2+} (s)
<i>Navicula sanctamargaritae</i>	0.13	-0.04	0.30	0.16	-0.41	0.15	0.12	0.07	-0.24	0.18	-0.30	-0.30	0.26	0.09	-0.31
<i>Surirella</i> sp1	-0.03	0.31	0.34	-0.16	-0.18	0.25	-0.07	-0.32	-0.17	-0.33	-0.23	-0.23	0.11	0.29	-0.22
<i>Unknown species1</i>	-0.18	-0.12	0.31	-0.17	-0.26	-0.03	-0.10	-0.13	0.12	-0.21	-0.21	-0.21	0.42	0.00	-0.22
<i>Planothidium frequentissimum</i> genetic variant A	-0.41	-0.43	-0.06	-0.36	0.41	-0.19	-0.36	-0.07	-0.07	-0.18	0.91	0.91	-0.26	-0.27	0.85
<i>Navicula veneta</i>	0.49	0.28	-0.21	0.58	0.68	0.20	0.44	0.46	0.39	0.76	-0.10	-0.10	-0.16	-0.07	-0.10
<i>Diploneis vacillans</i>	-0.20	-0.10	-0.06	-0.18	-0.22	-0.14	-0.19	-0.14	-0.11	-0.16	0.22	0.22	0.00	-0.05	0.32
<i>Gedaniella</i> sp.	-0.15	-0.24	-0.41	-0.16	0.45	-0.50	-0.03	0.12	-0.17	0.00	-0.08	-0.10	-0.22	-0.24	-0.09
<i>Pinnularia</i> sp1	0.07	0.41	-0.03	-0.07	-0.20	0.25	-0.06	-0.38	-0.22	-0.22	-0.16	-0.15	-0.12	0.40	-0.16
<i>Amphora copulata</i>	-0.27	-0.29	-0.13	-0.21	-0.21	-0.37	-0.29	-0.18	0.03	-0.14	0.32	0.31	0.13	0.13	0.43
<i>Planothidium frequentissimum</i> genetic variant B	-0.30	-0.40	-0.34	-0.16	-0.25	-0.51	-0.34	-0.46	-0.27	-0.17	-0.00	-0.02	-0.30	-0.24	0.01
<i>Unknown species2</i>	-0.14	-0.01	0.08	-0.06	-0.16	0.00	-0.11	-0.26	-0.25	-0.12	-0.13	-0.13	-0.17	-0.23	-0.15
<i>Pinnularia</i> sp2	0.60	0.41	-0.29	0.65	0.60	0.28	0.52	0.51	0.54	0.71	-0.13	-0.13	-0.20	-0.02	-0.13
<i>Pinnularia brebissonii</i>	0.50	0.33	-0.30	0.41	-0.19	0.27	0.49	0.65	0.83	-0.04	-0.11	-0.10	-0.19	-0.24	-0.12
<i>Gomphonema</i> sp1	-0.15	-0.26	-0.05	0.01	-0.22	-0.26	-0.15	-0.38	-0.22	-0.07	-0.15	-0.16	0.06	0.09	-0.20