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# ► To cite this version:

Lory-Anne Baker, Aude Beauger, Sofia Kolovi, Olivier Voldoire, 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-04033005

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.

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- 32
- 33 Abstract

Little is still known about the low dose effects of radiation on the microbial communities in the 34 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 distribution. Interestingly, <sup>238</sup>U was the second factor that explained diatom ASV structure. 51 52 Among the ASVs in the mineral springs monitored, ASV associated with one of the genetic variants of *Planothidium frequentissimum* was well represented in the springs and with higher 53 levels of <sup>238</sup>U, suggesting its high tolerance to this particular radionuclide. This diatom species 54 55 may therefore represent a bio-indicator of high natural levels of uranium.

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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 billion years, life has been evolving and adapting in ecosystems with different levels of natural 63 radioactivity (Martell, 1992; Garzón, 2001). Currently, there are regions on the Earth where 64 65 natural radioactivity can reach very high levels, such as India (Mohanty, 2004), Iran (Khodashenas, 2012) and Brazil (Oliveira, 1994). In Europe, median concentration of uranium 66 in soils is estimated to be around 2 mg.kg<sup>-1</sup>, with a range of variation from less than 0.1 to more 67 than 50 mg.kg<sup>-1</sup> (De Vos et al., 2006). In France, the highest concentrations are found in the 68 69 Massif Central (a mountain range in the middle of France), where it can reach hundreds of mg.kg<sup>-1</sup> in granitic soil (Garnier-Laplace, 2010). 70

71 In the Massif Central, several mineral springs show significant levels of natural radioactivity (Jacquet, 1929; Boineau, 1972; Risler, 1974; Ielsch, 2017). This natural radioactivity comes 72 from <sup>40</sup>K and the decay chains of three primordial radioelements <sup>235</sup>U, <sup>238</sup>U and <sup>232</sup>Th that are 73 naturally present in the Earth's crust, especially in granitic rocks (Métévier, 2001). Because of 74 its longer half-life (4,47 billion years), <sup>238</sup>U and its nuclear decay products (<sup>226</sup>Ra, <sup>222</sup>Rn, <sup>210</sup>Po) 75 are today the most dominant radionuclides in mineral springs (Erőss et al., 2018; Rihs et al., 76 77 2000; Morais et al., 2022). They are  $\alpha$ -particle emitters, the most mutagen ionising radiation involved in radioactive stress mainly related to internal irradiation in cells. Thus, mineral 78 springs in the French Massif Central are not only extreme environments due to their significant 79 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 laboratories' in which to study the influence of mineralisation and natural radioactivity on
biodiversity. These ecosystems are characterised by short food webs which could be
interesting to study the effects of natural radioactivity on primary producers. Diatoms are
part of the phytobenthos, and one of the main groups of primary producers in aquatic
ecosystems, including mineral springs. Algae are known to be good indicator for
radionuclides in aquatic environments and show differences in radionuclides tolerances
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 <sup>222</sup>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.

In the present study, and in order to bring new insights at both population and community levels, DNA metabarcoding was employed to assess the effect of enhanced natural radioactivity of water and sediments on benthic diatom communities in several mineral springs of the Massif Central. To this end, a 312 bp region of the chloroplast gene *rbc*L (encoding for the Ribulose Bisphosphate Carboxylase Large) was used as a barcode for diatom species identification (Vasselon, 2017), a convenient marker for such discriminations (Mann, 2010; Kermarrec, 2014; Bailet, 2020). Following on from earlier published results of Millan et al. (2019), where species richness was high even in radioactive mineral springs, our main working hypothesis was that radioactivity is not a major factor that influences diatom genetic richness. Furthrmore, because microbial species have different levels of resistance to ionising radiation (Shuryak, 2019), our second hypothesis was that radioactivity can influence diatom community structure because ionising radiation can shape the microbial community. In this light, a series of studies were performed in order to test these ideas empirically.

115 **2. Material and methods** 

### 116 2.1. Study sites and sampling

The sampling campaign took place in autumn from the 15<sup>th</sup> to the 30<sup>th</sup> October 2019, where 117 118 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 km<sup>2</sup>) 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

Benthic diatom samples were collected in the 16 mineral springs (Fig. 1). Since it was earlier demonstrated that the substrate has little influence on benthic diatom composition in mineral springs (Baker, 2022), the biofilm was recovered and considered to be the dominant substrate, 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

At the 16 spring sampling sites, water temperature (°C), conductivity (µS.cm<sup>-1</sup>) and pH (pH 138 139 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 of the following ions (expressed as  $mg.L^{-1}$ ): lithium (Li<sup>+</sup>), sodium (Na<sup>+</sup>), ammonium (NH<sub>4</sub><sup>+</sup>), 145 potassium ( $K^+$ ), magnesium ( $Mg^{2+}$ ), calcium ( $Ca^{2+}$ ), fluoride ( $F^-$ ), chloride ( $Cl^-$ ), nitrite ( $NO_2^-$ ), 146 nitrate (NO<sub>3</sub><sup>-</sup>) and phosphate (PO<sub>4</sub><sup>3-</sup>). 147

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

The activity of dissolved <sup>222</sup>Rn in water samples collected using 1 L Marinelli beakers was 149 150 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.

157 Analyses were conducted at Subatech (Nantes, France) and ICN (Nice, France) laboratories. 158 After PTFE filtration (0.45µm) and acidification of water samples (HNO<sub>3</sub> or HCl for Po 159 analysis), uranium was analysed by ICP-QMS (Xseries 2, Thermo Electron). Ultra-trace 160 concentrations of <sup>226</sup>Ra were measured without chemical purification by HR-ICP-MS in low-161 resolution mode (Element XR, Thermo Scientific) with an APEX-O high sensitivity desolvating 162 sample solution introduction system. In order to remove major polyatomic interferences from 163 the spectrum of <sup>226</sup>Ra 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 where major interferences can be resolved. The observed consistency of <sup>226</sup>Ra activity measured 166 167 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 characterised by a high sensitivity (4.7×105 CPS/Bq. mL<sup>-1</sup>) and a low instrumental detection 169 limit (0.8 mBq.L<sup>-1</sup>) for <sup>226</sup>Ra. Pb and Bi-based polyatomic interferences unresolved in low and 170 171 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, <sup>210</sup>Po 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).

Sediment samples were sieved to 2mm and dried at 105°C. <sup>226</sup>Ra and <sup>228</sup>Ac were analysed using a  $\gamma$ -Spectrometer with coaxial hyperpure Ge detector (GX4519, Mirion, >45% relative efficiency at 1.33 MeV between 10 keV to 10 MeV) according to NF EN ISO 18589-3. Measurements were conducted at least four weeks after the sealing of the sample container to ensure the secular equilibrium between <sup>226</sup>Ra, <sup>222</sup>Rn, <sup>214</sup>Pb and <sup>214</sup>Bi. The data were then processed via LabSOCS<sup>TM</sup> Calibration Software. <sup>238</sup>U, <sup>232</sup>Th and trace elements were measured by ICP-QMS (Xseries 2, Thermo Electron) after microwave digestion (Ethos Easy Milestone)
with concentrated HNO<sub>3</sub>, 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 *rbc*L 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).

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### 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.

The diversity data of all samples were exploited using the 'Phyloseq' package (McMurdie, 2013) in R version 4.1.2. The files obtained from the DADA2 data processing: ASVs table, taxonomy table and a table with the corresponding spring for each replicate were implemented in R to create a phyloseq object and facilitate data manipulation. Sample  $\alpha$ -diversity indices (ASV richness and Shannon–Wiener's (Hill, 1973) diversity index) were calculated for each 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, 2000a, 2000b, 2004; Krammer, 2000, 2002; Potatova and Hamilton, 2007; Lange-Bertalot
1979, 2001; Lange-Bertalot et al, 2011, 2017; Bey and Ector, 2013a, 2013b; Beauger et al,
2015; Levkov et al, 2016; Li et al, 2018).

#### 233 2.7. Statistical analysis

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

238 Different statistical analyses were perfored 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-mean)/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 >> 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.

The correlation between ASV richness and physico-chemical and radiological variables was tested by calculating Pearson correlation coefficient with XLSTAT 2020 version 5.1, to determine if these elements measured in our study influenced the number of diatom species in 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 vegedist of the R package "Vegan" (Oksanen et al., 2013, version 2.5-7). This index only takes into account the 260 261 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 function "dist.ldc" of the package "Adespatial" version 0.3-14 (Dray et al. 2012) was used to 263 264 generate a dissimilarity matrix with this second index. The individual influence of five environmental and radiological factors such as "Geographical location", "238U", "222Ra", 265 "<sup>210</sup>Po", <sup>228</sup>Ac" activities level on diatom community diversity in springs were determined 266 267 (Table S3) with a non-parametric MANOVA analysis [i.e. ADONIS (Permutational MANOVA)] based on Jaccard and "ab. Jaccard" dissimilarity indices using the function Adonis 268 269 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 variables (i.e. <sup>222</sup>Rn, <sup>238</sup>U, <sup>226</sup>Ra, <sup>210</sup>Po level, <sup>228</sup>Ac) were determined from the orders of 272 273 magnitude observed for these variables in the sediments of the sampled springs (Table 1). For  $^{238}$ U and  $^{226}$ Ra activities, three classes were determined by: Low: activity < 0.01 Bq.L<sup>-1</sup>; 274 Medium: 0.01 Bq.L<sup>-1</sup>  $\leq$  activity  $\leq$  0.1 Bq.L<sup>-1</sup>; High: activity > 0.1 Bq.L<sup>-1</sup> and Low: activity <275 0.1 Bq.g<sup>-1</sup>; Medium: 0.1 Bq.g<sup>-1</sup>  $\leq$  activity  $\leq$  1 Bq.g<sup>-1</sup>; High: activity > 1 Bq.g<sup>-1</sup> respectively. 276 Similarly, <sup>210</sup>Po activity levels were Low: activity < 0.00001 Bq.L<sup>-1</sup>; Medium: 0.00001 Bq.L<sup>-1</sup> 277

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

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282 Further correlations between the 14 most abundant species and physico-chemical and radiological variables were computed with XLSTAT 2020 version 5.1. The difference of ASV 283 284 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 different in species composition (here, predefined groups are geographical location and <sup>238</sup>U, 287  $^{226}$ Ra,  $^{210}$ Po,  $^{228}$ Ac activities). The computed MRPP statistic delta ( $\delta$ ) represents the overall 288 289 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

The physical, chemical, and radiological characteristics of the studied mineral spring sites are reported in Table 1. Conductivity of the mineral springs varied between 3090 and 9680  $\mu$ S.cm<sup>-1</sup> (mean of 5682.56 ± 1693.30  $\mu$ S.cm<sup>-1</sup>). pH did not display significant variations among the mineral springs (mean ± SD: 6.9 ± 0.3). The mean water temperature of the studied mineral 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<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> are high in mineral springs with an average of 939± 303 361 mg.L<sup>-1</sup> and 2318± 516 mg.L<sup>-1</sup> respectively. In the water, <sup>238</sup>U activity varied between 304 detection limit (2.5 mBq.L<sup>-1</sup>) and 0.193 Bq.L<sup>-1</sup> and <sup>210</sup>Po varied between 9 and 392 µBq.L<sup>-1</sup>. In 305 the sediments, <sup>226</sup>Ra activity variation extended on almost two orders of magnitude, from 0.56 306 to 52.1 Bq.g<sup>-1</sup>.Lastly, , considering all the springs, <sup>228</sup>Ac varied between detection limit (45 307 mBq.L<sup>-1</sup>) and 28.8 Bq.g<sup>-1</sup>.

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 <sup>226</sup>Ra activity is significant, include 312 Daguillon, Graviers, Ours, Tennis and Tête de lion. Also, Chemin, Graviers, Ours, Salut, Saulcée, Tennis and Tête de lion are represented by higher <sup>228</sup>Ac activity. Bard, Saladis, 313 314 Giraudon and Tennis springs show higher Ca<sup>2+</sup>, HCO<sub>3</sub><sup>-</sup> and K<sup>+</sup> concentrations. In the lower left quadrant, Estreys, Sail, Salins, Saulcée and Trois sauts show high <sup>210</sup>Po, <sup>238</sup>U activities and 315 Mn<sup>2+</sup> and PO<sub>4</sub><sup>3-</sup> concentrations. In the lower right quadrant, Giraudon, Rocs bleus, Saladis are 316 associated with higher temperature, conductivity, pH and DO and also with high Br<sup>-</sup> and Na<sup>+</sup> 317 318 concentrations.

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

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A total of 565 ASV were detected. Figure 3 shows the number of ASVs observed and Shannon's diversity index obtained for each mineral spring using the four technical replicates. The number of ASVs ranged from 6 and 74 with a mean  $29 \pm 17$ . The mineral spring with the most of ASVs was "Salut" (i.e.  $74 \pm 7$ ) and the lowest "Salins" (i.e.  $8 \pm 1$ ). In terms of Shannon's diversity index, the richest spring was "Chemin" (i.e.  $2.38 \pm 0.04$ ), the poorest "Tennis" (i.e.  $0.18 \pm 0.09$ ). There was no significant correlation between ASV richness and physico-chemical or
 radiological (<sup>238</sup>U, <sup>226</sup>Ra, <sup>210</sup>Po, <sup>228</sup>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 variability. For the radiological parameters, <sup>238</sup>U activities in the spring water explains 17.2 % 334 (Jaccard) and 38.6 % \* (ab.Jaccard) of the variability while its descendant <sup>226</sup>Ra activity in the 335 spring sediments explains 10.7 % (Jaccard) and 7.7 % (ab.Jaccard). <sup>210</sup>Po activities in the 336 springs' water contributes to 5.6 % (Jaccard) and 0 % (ab.Jaccard) of the variability while <sup>228</sup>Ac 337 activities in the springs sediments explains 13.3 % (Jaccard) and 3.5 % (ab.Jaccard) of this 338 339 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 Achnanthidium, Amphora, 344 Anomoeoneis, Caloneis, Craticula, Cyclotella, Diatoma, Diploneis, Epithemia, Fallacia, 345 Fistulifera, Fragilaria, Gedaniella, Gomphonema, Halamphora, Mayamaea, Navicula, Nitzschia, Pinnularia, Planothidium, Sellaphora, Surirella. The most dominant ASV (relative 346 347 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, some could not be assigned to a species and belong to the genera *Gomphonema*, *Navicula*, *Pinnularia* and *Surirella*. The other dominant assigned species are *Navicula sanctamargaritae*, *Gedaniella* sp., *Planothidium frequentissimum*, *Navicula veneta*, *Diploneis vacillans*, *Amphora copulata*, *Pinnularia brebissonii*, *Halamphora coffeaeformis*, *Gomphonema saprophilum*, and *Nitzschia vitrea*. Different ASVs were assigned to the same diatom species suggesting the 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<sup>+</sup> was correlated with *Pinnularia* sp2 ( $R^2=0.60$ ), which also correlated with several parameters as  $PO_4^{3-}$  (R<sup>2</sup>=0.60), conductivity (R<sup>2</sup>=0.52), pH (R<sup>2</sup>=0.51), DO (R<sup>2</sup>=0.54), 359 temperature ( $R^2=0.71$ ). Navicula veneta correlated with  $PO_4^{3-}$  (R=0.68) and temperature 360 361 (R<sup>2</sup>=0.76). Pinnularia brebisonii with pH (R<sup>2</sup>=0.65) and DO (R<sup>2</sup>=0.83). The genetic variant A of *Planothidium frequentissimum* correlated with <sup>238</sup>U activity in water (R<sup>2</sup>=0.91) and <sup>210</sup>Po 362 activity in water (R<sup>2</sup>=0.91), but no correlation was found for the genetic variant B. Additional 363 364 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 <sup>238</sup>U activity levels (in water) (with ab.Jaccard matrix: A=0.230, p=0.004). No significant 366 367 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 of *Planothidium frequentissimum* was associated with high level of <sup>238</sup>U (in water) (R<sup>2</sup>=0.998, 369 370 p=0.01).

### 371 **4. Discussion**

This study provides the first analysis of diatom communities living in mineral springs using DNA metabarcoding to assess the influence of enhanced natural radioactivity on these assemblages. These extreme aquatic environments in effect comprise a field laboratory in which to assess the contribution of natural radioactivity through radionuclides' activity on diatom community structure. From this, two hypotheses were posited: (1) that radioactivity is not a factor that particularly influences diatom genetic richness; and (2), that radioactivity is a factor that can influence diatom community structure.

379 4.1. Natural radioactivity did not impact ASV richness

380 A first parameter to describe the  $\alpha$ -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 between ASV richness and the various radiological parameters, viz. <sup>238</sup>U, <sup>226</sup>Ra, <sup>210</sup>Po, <sup>228</sup>Ac 383 384 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

Geographical location was found to be the main factor explaining diatom community structure in mineral springs considering ASV occurrence. This result is similar to those of Potapova and Charles (2002), Virtanen and Soininen (2012), Soininen et al. (2016) and Baker et al. (2021) that studied benthic diatom assemblages in rivers, streams and springs. <sup>238</sup>U activity was the second parameter that explained the variability of the diatom communities, whereas the variability of diatom community structure was more poorly explained by <sup>226</sup>Ra, <sup>210</sup>Po and <sup>228</sup>Ac 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 diatom community structure. Furthermore, bioaccumulation of <sup>226</sup>Ra by several organisms such 435 436 as plants, algae, bacteria, plankton and zooplankton is also documented to occur (Iyengar, 1990; 437 Zakeri, 2012; Carvalho, 2014; Heidari, 2017). <sup>226</sup>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 calcium in their metabolic processes calcium and therefore it is also likely that <sup>226</sup>Ra is 440 accumulated by diatoms and thereby involved in their metabolism too. <sup>210</sup>Po bioaccumulation 441 442 is well documented in marine environments (Bennett, 1979; Carvalho, 2018; Uddin, 2018), marine phytoplankton (Horowitz, 2020), and invertebrates from the Baltic sea (Skwarze and 443 444 Falkowski, 1988). All these radionuclides can be accumulated in cells of living organisms. Brown et al. (2004) observed that <sup>210</sup>Po and <sup>226</sup>Ra are the main contributors of absorbed dose 445 446 rates when they measured the internal concentration of several radionuclides within marine and freshwater organisms (e.g., phytoplankton, macrophytes). However, in our study, <sup>226</sup>Ra and 447 <sup>210</sup>Po are the two radionuclides that contribute the least to explanation of the community 448 449 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.

The correlations between the species associated to the 14 most abundant ASVs and 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 with high natural concentrations of the isotope <sup>238</sup>U. This result was confirmed with MRPP and 476 477 ISA, which revealed interestingly that this species is associated with high <sup>238</sup>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).

Mineral springs with high levels of radioactivity (i.e. <sup>238</sup>U activity) are habitats in which 486 487 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 diatom communities in the mineral springs, and <sup>238</sup>U the second factor. This radionuclide is 508 509 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.

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

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

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



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990 Figure 2: Principal component analysis (PCA) shows the springs' distribution based on physical, chemical and

<sup>991</sup> radiological variables measured in water (w) or sediments (s).



Figure 3: Boxplot showing the mean of (A) ASV richness corresponding to benthic diatom species richness and
(B) Shannon's diversity for each technical replicate (N=4) of the mineral springs monitored in the Massif Central
(France).



Figure 4: Taxonomic composition of diatom communities for the mineral springs monitored in the Massif Central
 based on DNA metabarcoding results for the *rbc*L chloroplast gene. Only species with reads relative abundances

above 1% are represented.

**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. <sup>238</sup>U; <sup>210</sup>Po <sup>226</sup>Ra; <sup>228</sup>Ac expressed radionuclides activity in spring water (w) or sediments (s).

Springs	Na <sup>+</sup> (mg L <sup>-1</sup> )	$\mathbf{K}^{+}$	$Ca^{2+}$ (mg L <sup>-1</sup> )	Br <sup>-</sup> (mg L <sup>-1</sup> )	$PO_4^{3-}$	HCO3 <sup>-</sup> (mg L <sup>-1</sup> )	Cond (uS cm <sup>-1</sup> )	рН	DO (%)	Temp (°C)	$^{238}U(w)$ (Ba L <sup>-1</sup> )	<sup>226</sup> Ra (s) (Ba g <sup>-1</sup> )	<sup>210</sup> Po (w) (uBa L <sup>-1</sup> )	$^{228}Ac(s)$ (Ba g <sup>-1</sup> )	$Mn^{2+}(s)$ (mg g <sup>-1</sup> )
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, <sup>238</sup>U; <sup>210</sup>Po <sup>226</sup>Ra; <sup>228</sup>Ac expressed radionuclides activity in spring water (w) or sediments (s). Values in bold are significant p-value ( $\alpha = 0.05$ ).

	ASV	Na <sup>+</sup>	<b>K</b> <sup>+</sup>	Ca <sup>2+</sup>	Br <sup>-</sup>	PO4 <sup>3-</sup>	HCO3 <sup>-</sup>	Cond	pН	DO	Temp	<sup>238</sup> U	<sup>226</sup> Ra	<sup>210</sup> Po	<sup>228</sup> Ac	Mn <sup>2+</sup>
												(w)	<b>(s)</b>	(w)	<b>(s)</b>	<b>(s)</b>
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 <sup>+</sup>	-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
<b>K</b> <sup>+</sup>	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 <sup>2+</sup>	-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
PO4 <sup>3-</sup>	-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
HCO3 <sup>-</sup>	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
рН	-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
<sup>238</sup> 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
<sup>210</sup> 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
<sup>226</sup> 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
<sup>228</sup> 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
<b>Mn</b> <sup>2+</sup> (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, <sup>238</sup>U, <sup>226</sup>Ra, <sup>210</sup>Po and <sup>228</sup>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	<b>R</b> <sup>2</sup> ( <b>J</b> )	<b>R</b> <sup>2</sup> ( <b>ab.J</b> )
Location	47.01 %	53.53 %
<sup>238</sup> U activity level (w)	17.18 %	38.59 % *
<sup>226</sup> Ra activity level (s)	10.69 %	7.74 %
<sup>210</sup> Po activity level (w)	5.64 %	0
<sup>228</sup> 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, <sup>238</sup>U, <sup>226</sup>Ra, <sup>210</sup>Po and <sup>228</sup>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). MRPP is based on 999 permutations. ASV: Amplicon sequence variant richness, Cond: Conductivity, DO: Dissolved oxygen, Temp: Temperature.

Distance index		Geographic location	<sup>238</sup> U Activity	<sup>226</sup> Ra Activity	<sup>210</sup> Po Activity	<sup>228</sup> Ac Activity
( <b>J</b> )	Chance-corrected within-group A	0.146	0.026	-0.049	-0.010	0.005
	Observed o	0.674	0.768	0.455	0.797	0.785
	Expected <b>d</b>	0.789	0.789	0.434	0.789	0.789
	Significance of <b>δ</b>	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 d	0.274	0.334	0.797	0.451	0.785
	Expected <b>\delta</b>	0.434	0.434	0.789	0.434	0.789
	Significance of <b>d</b>	p=0.11	p=0.004	p>0.05	p>0.05	p>0.05

Springs	Na <sup>+</sup> (mg.L <sup>-1</sup> )	K+ (mg.L <sup>-1</sup> )	Ca <sup>2+</sup> (mg.L <sup>-1</sup> )	Br <sup>-</sup> (mg.L <sup>-1</sup> )	PO4 <sup>3-</sup> (mg.L <sup>-1</sup> )	HCO <sub>3</sub> <sup>-</sup> (mg.L <sup>-1</sup> )	Cond (µS.cm <sup>-1</sup> )	pН	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 S1: Physical and chemical variables measured in the mineral springs monitored in the Massif Central (France). N=16

		Water		Sediments	
Springs	<sup>238</sup> U ( <b>Bq.L</b> <sup>-1</sup> )	<sup>226</sup> Ra (Bq.L <sup>-1</sup> )	<sup>210</sup> Po (µBq.L <sup>-1</sup> )	<sup>228</sup> Ac (Bq.g <sup>-1</sup> )	Mn <sup>2+</sup> (mg.g <sup>-1</sup> )
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 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 "<sup>226</sup>Ra (Bq.L<sup>-1</sup>)" specified that this variables could be measured for the sample.

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

Springs	Geographical location	<sup>238</sup> U level (w)	<sup>226</sup> Ra level (s)	<sup>210</sup> Po level (w)	<sup>228</sup> 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<sup>2+</sup>) 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 <sup>+</sup>	<b>K</b> +	Ca <sup>2+</sup>	Br <sup>.</sup>	PO.3-	HCO.	Cond	nH	DO	Temn	<sup>238</sup> U	<sup>210</sup> Po	<sup>226</sup> Ra	<sup>228</sup> Ac	Mn <sup>2+</sup>
	114		Ca		104	neos	Conu			remp	(w)	(w)	<b>(s)</b>	(s)	(s)
Navicula sanctamargaritae	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
Surirella 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
Unknown species1	-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
Planothidium frequentissimum 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
Navicula veneta	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
Diploneis vacillans	-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
Gedaniella 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
Pinnularia 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
Amphora copulata	-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
Planothidium frequentissimum 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
Unknown species2	-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
Pinnularia 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
Pinnularia brebissonii	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
Gomphonema 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