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1 **Microbial diversity alteration reveals biomarkers of contamination in soil-**  
2 **river-lake continuum**

3

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17

18 **Highlights:**

- 19 • Evidence of microbial community coalescence within a soil-river-lake continuum
- 20 • Specialist species identified are specifically associated to pollutant type
- 21 • Prediction of degradation functions of the microbial specialists
- 22 • New insights gained to develop biomonitoring tools for environmental pollution

23

24 **Abstract**

25 Microbial communities inhabiting soil-water-sediment continuum in coastal areas provide  
26 important ecosystem services. Their adaptation in response to environmental stressors,  
27 particularly mitigating the impact of pollutants discharged from human activities, has been  
28 considered for the development of microbial biomonitoring tools, but their use is still in the  
29 infancy. Here, chemical and molecular (16S rRNA gene metabarcoding) approaches were  
30 combined in order to determine the impact of pollutants on microbial assemblages  
31 inhabiting the aquatic network of a soil-water-sediment continuum around the Ichkeul Lake  
32 (Tunisia), an area highly impacted by human activities. Samples were collected within the  
33 soil-river-lake continuum at three stations in dry (summer) and wet (winter) seasons. The  
34 contaminant pressure index (PI), which integrates Polycyclic aromatic hydrocarbons (PAHs),  
35 alkanes, Organochlorine pesticides (OCPs) and metal contents, and the microbial pressure  
36 index microGAMBI, based on bacterial community structure, showed significant correlation  
37 with contamination level and differences between seasons. The comparison of prokaryotic  
38 communities further revealed specific assemblages for soil, river and lake sediments.  
39 Correlation analyses identified potential "specialist" genera for the different compartments,  
40 whose abundances were correlated with the pollutant type found. Additionally, PICRUSt  
41 analysis revealed the metabolic potential for pollutant transformation or degradation of the  
42 identified "specialist" species, providing information to estimate the recovery capacity of the  
43 ecosystem. Such findings offer the possibility to define a relevant set of microbial indicators  
44 for assessing the effects of human activities on aquatic ecosystems. Microbial indicators,  
45 including the detection of "specialist" and sensitive taxa, and their functional capacity, might  
46 be useful, in combination with integrative microbial indices, to constitute accurate  
47 biomonitoring tools for the management and restoration of complex coastal aquatic  
48 systems.

49

50 **Keywords:** Aquatic ecosystems; Environmental pollution; Environmental monitoring;  
51 Microbial Ecology; Community structure; Functional potential

## 52 INTRODUCTION

53 Most human activities concentrate around the aquatic environments such as coastal areas,  
54 estuaries, lagoons, lakes and rivers (Solomon 2010). Thus, these aquatic environments and  
55 their surroundings are threatened by chemical pollution from urbanization, agriculture,  
56 industry and recreational activities (Landrigan et al., 2018). Once in the environment, many  
57 contaminants persist for a long period of time, resulting in chronic pollution, especially  
58 polycyclic aromatic hydrocarbons (PAHs), pesticides and metals (Ben Salem et al., 2016a;  
59 Bordenave et al., 2004a; Bruneel et al., 2008; Mwanamoki et al., 2014). Therefore, careful  
60 monitoring is required to evaluate the ecological status of aquatic ecosystems (Birk et al.,  
61 2012; Poikane et al., 2020).

62 Microbial communities play a crucial role in ecosystems functioning, particularly in organic  
63 matter decomposition, nutrients cycling and pollutants removal (Bordenave et al., 2008;  
64 Duran and Cravo-Laureau 2016; Duran et al., 2015b; Giloteaux et al., 2010; Guermouche  
65 M'rassi et al., 2015). The development of ecotoxicological tools and ecological indices based  
66 on microbial community structure and composition have therefore been proposed to  
67 evaluate the environmental health (Aylagas et al., 2017; Chen et al., 2019; Cordier et al.,  
68 2017, 2019; Cravo-Laureau et al., 2017; Lanzén et al., 2020; Xie et al., 2018). Several studies  
69 have demonstrated the effect of contaminants on microbial community structure,  
70 composition and diversity (Bordenave et al., 2004b; Borja 2018; Stauffert et al., 2014). Such  
71 studies have revealed the links between microorganisms and their environment (Barberán et  
72 al., 2012; Misson et al., 2016; Yergeau et al., 2012), allowing the identification of possible  
73 pollutant degraders as well as sensitive microorganisms to specific pollutant compounds  
74 (Duran et al., 2015a; Jeanbille et al., 2016a,b; Yergeau et al., 2012), which represent useful  
75 information for environmental health monitoring. However, terrestrial aquatic ecosystems  
76 are complex hydrologic networks where different environmental compartments (i.e.  
77 surrounding soil, river, lake and marine water) are connected, corresponding to a patchwork  
78 of habitats (Ruiz-González et al., 2015). Precipitation runoff is often the main way that  
79 connects these environmental compartments within a soil-river-lake continuum that  
80 contributes to the transferring organic and inorganic materials from the soil through the soil  
81 to water and to the sediment. Such connectivity results in microbial community coalescence,  
82 the arrival of microorganisms together with particulate organic matter from a habitat to

83 another, reorganizing the microbial community (Luo et al., 2020; Rillig et al., 2015). Microbial  
84 community coalescence relies on the "metacomunity" concept that considers a microbial  
85 community occupying a specific habitat as part of a larger community differentially adapted  
86 according to the environmental conditions (Stendera et al., 2012). It is therefore of  
87 paramount importance to consider the whole networks (continuum soil-river-lake) in order  
88 to fully understand the microbial community assemblage rules in aquatic ecosystems,  
89 particularly the role of pollutants in the organization of microbial communities. However,  
90 few studies have considered the aquatic environment as a whole (Luo et al., 2020; Ruiz-  
91 González et al., 2015). As a result, our knowledge on the effect of pollutants on microbial  
92 assemblages in aquatic networks remains limited.

93 Several studies have reported the use of microbial "specialist" as pollution bioindicators,  
94 such as *Oleispira* and other hydrocarbon-degrading bacteria for reporting petroleum  
95 contamination in seawater (Krolicka et al., 2019), and *Bacteroidia* and *Nitrospira* for metal  
96 contamination in wetland sediments (Li et al., 2020a). Despite that the soil-river-lake  
97 continuum represents a mixing zone, we hypothesize that compartment fragmentation will  
98 allow to identify specific microbial "specialist" that can serve as pollution bioindicators in  
99 coastal areas. In order to test this hypothesis, microbial communities were characterized in  
100 transects along soil-river-lake continuum located in three stations around the Ichkeul Lake  
101 (Tunisia). The Ichkeul Lake, connected to the Bizerte Lagoon, is a receptacle of rain winter  
102 runoffs from various oueds (wadi) watersheds (Ben Salem et al., 2016a, 2017) located in a  
103 region with intense human activities including agriculture, mining, oil refinery and  
104 metallurgy (Ben Salem et al., 2017). It is thus a hydrologic network contaminated with  
105 multiple compounds (Ben Said et al., 2015; Ben Salem et al., 2016a,b, 2017, 2019), which  
106 allows to study the effect of pollutants in different compartments along soil-river-lake  
107 continuums. The microbial communities were characterized by 16S rRNA gene barcoding to  
108 decipher the effects of pollutants on community assemblage in soil-river-lake continuum at  
109 three stations located around the Ichkeul Lake. The microbial community composition in the  
110 different compartments of the soil-river-lake continuum was examined at the dry (summer)  
111 and wet (winter) seasons in order to depict the spatiotemporal variations. The correlation of  
112 microbial community composition with pollutant contents identified microbial "specialists"  
113 specifically abundant in presence of a pollutant.

## 114 MATERIALS AND METHODS

### 115 *Site description*

116 Ichkeul Lake (North Tunisia, Fig. 1) is registered in several international conventions,  
117 including the UNESCO Biosphere Reserve and World Heritage (1977), and RAMSAR (1980).  
118 Ichkeul Lake has an area of 85 km<sup>2</sup> with a depth average varying between 1 m (summer) and  
119 2 m (winter). It receives waters from runoff via several wadis, streams with rainfall regimes.  
120 Its hydrological flow, due to its connection with the Bizerte Lagoon, results in salinity  
121 variations according to seasons (Ben Salem et al., 2017). The salinity fluctuation contributes  
122 to maintain an important level of biodiversity from microorganisms (Ben Salem et al., 2019)  
123 to higher macroorganisms such as birds (Ramdani et al., 2001) and fishes (Gharbi-Bouraoui  
124 et al., 2008). Because it is an area concentrating diverse human activities (e.g. intensive  
125 farming, petroleum refinery, mining and metallurgy), the Ichkeul Lake receives various  
126 contaminants including pesticides, petroleum hydrocarbons, organic chlorinated compounds  
127 (OCPs) and metals, principally from winter rains runoffs via the wadis (Ben Salem et al.,  
128 2016a).

129

### 130 *Sampling*

131 In order to characterize the effect of pollutants on microbial communities along the soil-  
132 river-lake continuums, three stations were selected according to their location considering  
133 surrounding human activities resulting in contrasted pollutions. The stations S2 and S3 are  
134 located in a cereal culture area where there is an intensive use of pesticides, while the  
135 station S6, located in a vegetable crops area where pesticides are used in lower amounts.  
136 Additionally, the station S2 is located close to a refinery and urban area (Ben Salem et al.,  
137 2017), whereas the station S3 is threatened by metal pollutants coming from acid mine  
138 drainages located upstream in the former Jalta lead mine area (Ouchir et al., 2016).  
139 Consistently, a previous study reported contrasting contamination levels of petroleum  
140 hydrocarbons and pesticides in the lake sediments at the three stations. S2 had high  
141 concentrations of petroleum hydrocarbons and pesticides, while S3 had high pesticide and  
142 metal concentrations together with low petroleum hydrocarbons concentration, and S6 had  
143 opposite trend to S3 (Ben Salem et al., 2017).

144 Transects along the soil-river-lake continuum were sampled at the three stations (S2, S3 and  
145 S6) located around the Ichkeul Lake (Fig. 1). The soil samples were collected in agricultural  
146 field located about 50 - 800 m upstream the river sediments (Fig. 1). The lake sediments  
147 were collected at the mouth of the river where the river sediments were sampled, about 300  
148 – 2200 m downstream (Fig. 1). Soil, river and lake sediments were collected at the dry  
149 (summer, September 2018) and wet (winter, April 2019) seasons, in true biological triplicate.  
150 Thus, 54 samples (3 replicates x 3 compartments x 3 stations x 2 seasons) were obtained.

151 The surface of the soil and the river sediments were collected manually with a sterile  
152 spatula. The surface sediments of lake were collected with a 200 cm<sup>2</sup> Van Veen grab as  
153 previously described (Ben Salem et al., 2016b). The samples were dispatched in 2 mL sterile  
154 cryotubes frozen quickly in liquid nitrogen for microbial analysis, and in 25 mL tubes  
155 maintained at 4°C for chemical analysis. Samples for microbial analyses were conserved at -  
156 80°C, and those for chemical analyses at -20°C until analysis.

157

#### 158 ***Physical-chemical analysis and anthropogenic pressure indices***

159 Chemical analyses for OCPs, PAHs and alkanes were performed using the QuEChERS method  
160 (Ben Salem et al., 2016b) followed by gas chromatography-mass spectrometry (GC-MS) as  
161 previously described (Yang et al., 2010).

162 Deuterated alkanes (nonadecane C<sub>19</sub>d<sup>40</sup> and triacontane C<sub>30</sub>d<sup>62</sup> at 10 mg mL<sup>-1</sup>) and PAH  
163 (naphthalene-d<sup>8</sup> and anthracene-d<sup>10</sup> at 10 mg mL<sup>-1</sup>) internal standards were added to each  
164 sample and quantification was performed on an Agilent 7890A Gas Chromatography system  
165 coupled with a Turbomass Gold Mass Spectrometer with Triple-Axis detector, operating at  
166 70 eV in positive ion mode. External multilevel calibrations were carried out using alkanes  
167 [Standard Solution (C<sub>8</sub>–C<sub>40</sub>); Sigma], methylated-PAHs (1-methylnaphthalene, 2-  
168 methylanthracene, and 9,10-dimethylanthracene; Sigma), and PAH (QTM PAH Mix; Sigma)  
169 standards, the concentrations of which ranged from 1.125 to 18 mg mL<sup>-1</sup>. For quality control,  
170 a calibration standard (10 mg mL<sup>-1</sup>) and a blank were analysed every 10 samples. We  
171 quantified alkanes between C<sub>10</sub> and C<sub>36</sub> including pristane and phytane and the following  
172 PAHs: naphthalene; all isomers of methyl-, dimethyl- and trimethyl-naphthalenes;  
173 acenaphthylene; acenaphthene; fluorene; phenanthrene; all isomers of methyl- and  
174 dimethylphenanthrenes/ anthracenes; fluoranthene; pyrene; all isomers of methyl- and



175 dimethyl-pyrene; chrysene; all isomers of methyl and dimethyl-chrysene,  
176 Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Indeno[1,2,3-cd]pyrene,  
177 Dibenzo[a,h]anthracene and Benzo[g,h,i]perylene.

178 For the OCP, quantitative analysis was carried out using selected ion monitoring mode (SIM)  
179 as described by Yang et al (2010). The standard of organochlorine pesticide mixture from  
180 Supelco consisted of a solution in n-hexane–toluene (1:1, v/v) at 2,000  $\mu\text{g mL}^{-1}$  of  $\alpha$ -  
181 hexachlorocyclohexane ( $\alpha$ -HCH),  $\beta$ -hexachlorocyclohexane ( $\beta$ -HCH),  $\gamma$ -  
182 hexachlorocyclohexane ( $\gamma$ -HCH),  $\delta$ -hexachlorocyclohexane ( $\delta$ -HCH), endosulfan  $\alpha$ ,  
183 endosulfan  $\beta$ , endosulfan sulfate, aldrin, dieldrin, endrin, endrin aldehyde, endrin ketone,  
184 heptachlor, heptachlor epoxide, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane, (4,4'-DDT),  
185 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane (4,4'-DDD), 1,1-dichloro-2,2-bis(4-  
186 chlorophenyl)ethylene (4,4'-DDE), methoxychlor, and hexachlorobenzene (HCB). A standard  
187 solution of 2,4,5,6-tetrachloro-*m*-xylene and dibutylchlorodate in n-hexane–toluene (1:1,  
188 v/v) at 2,000  $\text{mg mL}^{-1}$  was purchased from Supelco to use as internal standard. The  
189 concentration range of each pesticide was from 0.15 to 2  $\mu\text{g L}^{-1}$  and the internal standards  
190 used in quantification at a concentration of 1  $\mu\text{g L}^{-1}$ .

191 In order to determine the origin of the PAH contamination in sediments, the PAH isomer  
192 ratio FLA/(FLA+PYR), where FLA: fluoranthene and PYR: pyrene, was calculated. The  
193 FLA/(FLA+PYR) ratio was used because it is a more conservative ratio than the other  
194 diagnostic ratios, being less sensitive to photodegradation (Tobiszewski and Namieśnik  
195 2012). Thus, it is suitable for the identification of PAH sources in soil and sediment  
196 samples receiving indirect PAH inputs (Tu et al., 2018). The FLA/(FLA+PYR) ratio below 0.4  
197 suggests a petroleum input, the ratio between 0.4 and 0.5 indicates a combustion of fossil  
198 fuel, and the ratio above 0.5 signifies a biomass combustion (De La Torre-Roche et al., 2009).

199 Metal concentrations including Cr, Cu, Ni, Pb, Zn, As and Cd, were determined using an  
200 inductively coupled plasma optical emission spectrometry (ICP-EOS; ISO 11885: 2007) and  
201 mercury was detected by atomic absorption spectrophotometry (AAS) as described in EPA  
202 Method 7473 (SW-846). The physical-chemical parameters are summarised in supplementary  
203 material (Table S1).

204 Pressure index (Pi) was calculated as previously described by Aylagas et al. (2017), although  
205 redox potential and PCB were not measured and thus not considered. In comparison to  
206 other indexes to estimate the potential toxic effect of pollutants on ecosystem, the Pi has

207 the advantage to be integrative. It is based on the average of normalized threshold effects  
208 levels (TEL) for each individual pollutant considered (OCPs, PAHs, alkanes, and metals)  
209 effective to estimate the toxic effect of pollutants in aquatic ecosystem (Aylagas et al.,  
210 2017).

211 The threshold values used for determining the Pi were based on the TEL from the National  
212 Oceanic and Atmospheric Administration (NOAA 1999) presented in supplementary Table  
213 S2. Pi values range between 0 and 5 where 0 corresponds to a total absence of pollutant  
214 pressure and 5 corresponds to the maximum pressure. The ecological status was evaluated  
215 using the microbial genomic AZTI's Marine Biotic Index (microgAMBI index), calculated as  
216 previously reported (Aylagas et al., 2017; Borja 2018).

217

### 218 ***DNA extraction, PCR amplification and Sequencing***

219 Total DNA was extracted from 250 mg of frozen soil and sediment samples after grinding  
220 with liquid nitrogen, using the PowerSoil DNA extraction kit (MoBio Laboratories Inc.),  
221 following the manufacturer's instructions with minor modifications as previously described  
222 (Giloteaux et al., 2013). PCR amplifications were performed using universal prokaryotic  
223 primers U515-532-GTGYCAGCMGCCGCGGTA and U909-928-CCCCGYCAATTCMTTTRAGT  
224 targeting the V4-V5 region of the 16S rRNA gene (Wang and Qian 2009). Each sample was  
225 amplified in triplicates in a total volume reaction of 25  $\mu$ L, using 12.5  $\mu$ L of AmpliTaq Gold  
226 360 Master Mix, 0.5  $\mu$ L of each forward and reverse primers (20  $\mu$ M), and 5  $\mu$ L (10 ng) of the  
227 DNA extracts. The final volume was adjusted with distilled water. Thermal cycling was  
228 carried out under the following conditions: 95  $^{\circ}$ C for 10 min, 30 cycles of 95  $^{\circ}$ C for 30 s, 60  $^{\circ}$ C  
229 for 30 s, 72  $^{\circ}$ C for 40 s, and a final extension step at 72  $^{\circ}$ C for 10 min. For each sample, the  
230 three PCR products were pooled and sequenced by Illumina-MiSeq (paired-end 2 x 250 bp)  
231 at the GenoToul platform (Toulouse, France).

232

### 233 ***Sequence data analysis***

234 Read-pair overlapping, sequence data filtering and clustering into SWARM Operational  
235 Taxonomical Units (OTUs), was done for each sample as previously described by Lanzén et  
236 al. (2020). Briefly, reads were overlapped using *vsearch* v2.7.1 (Rognes et al., 2016). Primers  
237 were then removed using *cutadapt* v1.15 (Martin 2011). Remaining sequences were de-

238 replicated and sorted by abundance using *vsearch*. SWARM v2.2.1 (Mahé et al., 2015) was  
239 then used to cluster the reads into OTUs with a minimum linkage of one nucleotide (similarly  
240 to sequence variants rather than traditional maximum linkage clustering). Abundances of  
241 unique sequences across samples were retained and used to construct an OTU contingency  
242 table based on SWARM output, by using SLIM (Dufresne et al., 2019). We discarded  
243 singleton OTUs and thereafter applied reference-based and *de novo* UCHIME chimera  
244 filtering as implemented in *vsearch* (Lanzén et al., 2012), with SilvaMod v128 as reference  
245 database (<https://github.com/lanzen/CREST>). We then applied LULU post-clustering curation  
246 with a minimum 97% similarity cut-off (Frøslev et al., 2017) to correct remaining artefacts  
247 and merge OTUs with intra-specific or intra-genomic differences. Final SWARM OTUs were  
248 aligned to SilvaMod v128 using *blastn* v2.6.0+ and taxonomically classified using CREST  
249 v3.1.0 (Lanzén et al., 2012). In all samples high sequencing coverage was obtained as shown  
250 on the rarefaction curves (supplementary material, Fig. S1). The complete dataset was  
251 deposited in the NCBI Sequence Read Archive (SRA) database (SUB8782050). It is available  
252 under the Bioproject ID PRJNA688542.

253 Predicted cross-contaminant reads were removed using a procedure analogous to UNCROSS  
254 (Edgar 2016) by setting sample-specific OTU abundances to zero when encountered at an  
255 abundance below 2% of the average OTU abundance across samples. Further, in order to  
256 compensate for the possible bias introduced by uneven sequencing depths, OTUs present  
257 with a maximum abundance across samples below 0.01%, were discarded as previously  
258 proposed (Elbrecht et al., 2018).

259 Functional prediction of microbial communities was determined using PICRUSt2  
260 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States, version  
261 2), allowing to infer the functions encoded in bacterial communities from the 16S rRNA gene  
262 sequences (Douglas et al., 2020). The Kyoto Encyclopedia of Genes and Genomes (KEGG)  
263 databases (e-value cut-off  $10^{-5}$ ) were used for functional annotation and metabolism  
264 analyses, as previously described (Liu et al., 2018). In order to assess the accuracy of PICRUSt  
265 analysis, the weighted nearest sequenced taxon index (NSTI) was calculated (Langille et al.,  
266 2013).

267

268

269

## 270 ***Statistical analysis***

271 Statistical analyses were performed using R packages vegan (Oksanen et al., 2013). Alpha  
272 diversity indices of microbial community were calculated based on the rarefied OTU table.  
273 The differences between these indices were tested by analysis of variance (ANOVA).  
274 Differences among group means were tested using least significant difference test LSD,  
275 function LSD.test (used for multiple comparisons) within “agricolae” package after ANOVA  
276 test. Permanova (permutational multivariate analysis of variance) was performed to  
277 estimate the effect of factors and their interactions on the microbial communities (999  
278 permutations). ANOSIM (analysis of similarities) was performed to compare samples.  
279 SIMPER analyses based on Bray-Curtis similarity matrix were carried out to determine the  
280 OTUs contribution and their differences between sites, stations and seasons. Venn diagram  
281 was visualized with InteractiVenn (Heberle et al., 2015), based on the distribution of OTUs  
282 showing specific and shared OTUs between sites, stations and seasons.  
283 Community similarity was assessed by Non-Metric multidimensional Scaling (NMDS)  
284 correlating prokaryotic communities with physical-chemical parameters and chemical  
285 compounds. The sum of the different type of contaminants ( $\sum$  alkanes,  $\sum$  PAHs,  $\sum$  Metals and  
286  $\sum$  OCPs), each individual metal, and Pi were used in order to consider the multi-  
287 contamination for each type contaminant. The significant chemical variables were fitted to  
288 the NMDS as vectors with the envfit function on R Statistics software.  
289 Linear discriminant analysis effect size (LEfSe) (Segata et al., 2011) was applied to determine  
290 seasons and sites specific biomarker. It performs several tests to assess their reliability.  
291 Firstly, a Kruskal-Wallis (KW) sum rank non-parametric test was performed to detect  
292 significant differential taxa abundance ( $p < 0.05$ ). A pairwise Wilcoxon test was performed to  
293 investigate the biological consistency ( $p < 0.05$ ). The linear discriminant analysis (LDA)  
294 logarithmic threshold was setup to 2. A heatmap-clustering correlation was performed with  
295 CIMminer (<http://discover.nci.nih.gov/cimminer/>). These analyses were performed at the  
296 genus level to determine the relation between pollutants concentrations and taxon  
297 abundances and genus rank.

298

299

300

## 301 RESULTS AND DISCUSSION

### 302 *Contamination level*

303 The principal component analysis (PCA), based on physical-chemical parameters  
304 (granulometry, ions, C and N contents...), indicated that the compartments (soil, river, and  
305 lake) shared similar characteristics (supplementary material, Fig. S2 and Table S1),  
306 suggesting limited influence of physical-chemical parameters in shaping the microbial  
307 community structure. Such observation indicated a soil-river-lake continuum with exchange  
308 of material probably via winter rain runoffs as the Ichkeul Lake is a receptacle of the  
309 surrounding watersheds (Ben Salem et al., 2016a). Accordingly, the PCA also distinguished  
310 lake in summer by high content of chloride and sodium ions, while most soil in winter were  
311 rich in carbonate (supplementary material, Fig. S2 and Table S1). Such result was not  
312 surprising since the Ichkeul Lake receives runoff inputs from winter rains while the water  
313 level decreases in summer increasing salinity in summer (dry season). From the physical-  
314 chemical analyses (supplementary material, Table S1) for samples collected in September  
315 2018 (summer, dry season) and in April 2019 (winter, wet season) at the three stations (S2,  
316 S3, and S6) the sums of the different types of pollutants were calculated (Table 1). All the  
317 stations contained higher metal concentrations than PAHs, alkanes and OCPs. The metal  
318 concentrations ranged between  $300 \pm 55$  to  $2421 \pm 2457$  mg kg<sup>-1</sup> in summer and  $217 \pm 20$  to  
319  $2536 \pm 677$  mg kg<sup>-1</sup> in winter. Metal concentrations were significantly different between  
320 compartments (two-way ANOVA,  $p < 0.001$ ), but not between seasons ( $p > 0.05$ ). In  
321 contrast, the organic contaminants (PAHs, Alkanes and OCPs) showed different  
322 concentrations according to compartments (two-way ANOVA,  $p < 0.001$ ) and seasons  
323 (two-way ANOVA,  $p < 0.01$ ). Total PAHs concentrations ranged from  $288 \pm 4$  to  $1210 \pm 20$  ng  
324 g<sup>-1</sup> and from  $322 \pm 4$  to  $1436 \pm 26$  ng g<sup>-1</sup> in summer and winter respectively, being higher in  
325 winter than in summer (two-way ANOVA,  $p < 0.001$ ), while the alkane concentrations were  
326 higher (two-way ANOVA,  $p < 0.001$ ) in summer (from  $193 \pm 8$  to  $637 \pm 10$  ng g<sup>-1</sup>) than in  
327 winter (from  $301 \pm 27$  to  $1127 \pm 63$  ng g<sup>-1</sup>). The OCP concentrations showed similar trend as  
328 alkane concentrations, being higher in summer than in winter (two-way ANOVA,  $p < 0.01$ ).  
329 The concentrations of organic contaminants (PAHs, Alkanes and OCPs) were higher in  
330 soil than in river and lake sediments (two-way ANOVA,  $p < 0.001$ ), while the metal  
331 concentrations were higher in river and lake sediments than in soil (two-way ANOVA,  $p <$

0.01). Also, the contaminant concentrations were different between river and lake sediments (two-way ANOVA,  $p < 0.001$ ). Such observations correlated with the on-going anthropogenic activities taking place around the Ichkeul Lake, such as the presence of an oil refinery near S2 (Ben Said et al., 2010), intensive agriculture practices near S3 (Stevenson and Battarbee 1991) and a wastewater discharge from an iron mine plant in an upstream location (Touaylia et al., 2016). Accordingly, the ratio FLA/(FLA+PYR) was found to be between  $0.4 \pm 0.01$  and  $0.8 \pm 0.1$  indicating the pyrogenic origin of PAHs by combustion of both petroleum and biomass, as previously described (De La Torre-Roche et al., 2009). Although the diagnostic ratio should be considered with caution, the FLA/(FLA+PYR) ratio, suitable for the identification of PAH sources in soil and sediments (Tu et al., 2018), further demonstrated the human impact on the Ichkeul Lake area. In accordance with the PAH concentrations trend, the FLA/(FLA+PYR) ratio showed differences according to compartments (two-way ANOVA,  $p < 0.001$ ) but not to seasons (two-way ANOVA,  $p > 0.1$ ). In order to estimate the toxic effect of pollutants on the ecosystem, the Pressure index (PI), integrating the toxic effect of all individual pollutants (Aylagas et al., 2017), was calculated. The Pi values of the compartments (soil, river and lake from each station) also indicated a moderate pressure (Table 1). The Pi values were different according to compartments (two-way ANOVA,  $p < 0.01$ ) and seasons (two-way ANOVA,  $p < 0.05$ ). The soil at station S3 in winter was the most contaminated ( $Pi = 2.93 \pm 0.15$ ), while the sediment river at station S6 in summer was the less ( $Pi = 1.46 \pm 0.2$ ). Although the contamination level was slightly divergent in all sites, it is interesting to note that all sites showed a similar trend based on the Pi values as follows: sediment river > sediment lake > soil (Table 1).

354

### 355 *Prokaryotic community structure and composition*

356 The comparison of the microbial communities at each station allowed to determine whether  
357 the environmental pressure exerted at each site had a specific influence on the microbial  
358 organisation. A total of 641,382 reads (410 bp) were obtained. After trimming 13,509  
359 sequences were retained per sample and dispatched within 2,250 OTUs, which were  
360 grouped into 26 phyla (Fig. 2).

361 All stations, irrespective of the compartments, were dominated by Proteobacteria,  
362 Actinobacteria, Bacteroidetes, and Chloroflexi (Fig. 2). These phyla have been detected as

363 dominant in Ichkeul Lake/Bizerte Lagoon hydrological system (Ben Salem et al., 2019) and in  
364 diverse environments, including river sediments (Sun et al., 2013) and soils (Lin et al., 2019).  
365 They have been described playing a key role in biogeochemical cycles (Andrews et al.,  
366 2011) and in contaminant degradation, including petroleum hydrocarbons (Guermouche  
367 M'rassi et al., 2015) and pesticides (Magnoli et al., 2020). The microbial community  
368 composition of the soil samples appeared similar regardless of the season (ANOSIM,  $p >$   
369  $0.05$ ) but differed according to the stations (ANOSIM,  $p < 0.01$ ). In contrast, the composition  
370 of the river and the lake sediments shifted between summer and winter (ANOSIM,  $p < 0.05$ ).  
371 Noteworthy, the lake sediments showed specific patterns in summer (dry season) that were  
372 different (ANOSIM,  $p < 0.01$ ) to those observed in winter (wet season, Fig. 2). Nonetheless,  
373 microgAMBI showed similar ecological status, ranging from good to moderate, as shown by  
374 the Pi (Table 1 and 2), revealing that bacterial communities responded to the pollution levels  
375 as detected by Aylagas et al. (2017). The comparison of alpha diversity indices did not reveal  
376 significant differences (Two-way ANOVA,  $p > 0.05$ ) between the samples (Table 2). The  
377 adaptation of the microbial communities to pollutants (legacy effect) and their mixing  
378 (coalescence) may explain the fact that microbial diversity was similar in all samples, in line  
379 with previously reported results (Jurgburg et al., 2017; Nunes et al., 2018; Yin et al., 2015).

380 The comparison of the microbial communities by NMDS analysis separated most of the soil,  
381 river sediment and lake sediment into distinct compartment-specific clusters (Fig. 3A),  
382 highlighting their different composition. Such observation was further supported by  
383 Permanova showing that the microbial diversity variation was explained mainly by the  
384 compartment origin ( $p < 0.002$ ; Fig 3B), the bacterial communities from soil being  
385 significantly different from that of sediments (Permanova,  $p < 0.002$ ). The bacterial  
386 communities of river sediment showed significant difference with that of lake sediment  
387 (Permanova,  $p < 0.01$ ). However, the microbial communities of river sediments from S2 in  
388 summer was clustered far from other river sediment samples, close to lake sediments. This  
389 may be explained by the fact that the sampling sites in river and lake were closer at site S2  
390 (Fig. 1). Nevertheless, the soil cluster was closer (Fig. 3A; ANOSIM,  $p < 0.001$ ) to the river  
391 sediment cluster (SIMPER: 26 %) than that of the lake sediment (SIMPER: 5 %), consistent  
392 with the soil-river-lake continuum. When comparing communities between the two seasons,  
393 differences were observed within the environmental compartments (Permanova,  $p < 0.01$ ),

394 the lake cluster being the more dispersed, particularly for stations S2 and S3 (Fig. 3). This  
395 confirms the seasonal variations of communities observed in the Ichkeul Lake sediments  
396 (Ben Salem et al., 2019). It is also consistent with the physical-chemical variations observed  
397 in the Ichkeul Lake (supplementary material, Table S1), showing salinity modification  
398 according to the hydrology of the Ichkeul Lake receiving saline water from Bizerte Lagoon in  
399 summer (Ben Salem et al., 2017). It has been demonstrated that salinity is a main driver for  
400 community organization (Xi et al., 2014), with its fluctuation drastically affecting microbial  
401 community composition and structure (Ben Salem et al., 2019). The NMDS analysis  
402 substantiated the capacity of Pi and microgAMBI to reveal an agreement between the  
403 ecological status (good and moderate) and the level of pressure (low-moderate; Tables 1  
404 and 2), indicating that lake sediment was the more exposed to contaminants than river  
405 sediment and soil (Fig. 3). Consistently, the NMDS showed that the Total PAHs ( $\Sigma$  PAH)  
406 influenced the microbial communities of the lake sediment. This agrees with previous  
407 reports showing that this contaminant affects microbial communities in diverse  
408 environments (Giloteaux et al., 2010, 2013; Vercraene-Eairmal et al., 2010), especially in  
409 coastal areas (Duran et al., 2015a,b; Pringault et al., 2008). Consistently, the Venn diagram  
410 (supplementary material Fig. S3) showed an average of  $41.3 \pm 3.73$  shared OTUs between  
411 compartments (soil, river and lake) in each station (S2, S3 and S6) considering both seasons.  
412 These shared OTUs correspond to the core community at the stations, further confirming  
413 the connectivity along the soil-river-lake continuum. These observations were supported by  
414 SIMPER analyses, especially for the core community composition (SIMPER: 10 %). The Venn  
415 diagram also showed a core community for each season (supplementary material Fig. S3),  
416 more important in winter  $24 \pm 22$  OTUs (SIMPER: 34 %) than in summer,  $1.3 \pm 1.2$  OTUs  
417 (SIMPER: 34 %). The seasonal comparison of the communities in the compartments (soil,  
418 river and lake) revealed seasonal stable OTUs (shared OTUs by a compartment irrespective  
419 of the season), which number was higher in soil  $960 \pm 145$  OTUs (SIMPER: 47 %), than in  
420 both aquatic ecosystems, lake  $716 \pm 533$  OTUs (SIMPER: 44 %) and river  $471 \pm 388$  OTUs  
421 (SIMPER: 42 %) indicating that soil represents a habitat where communities are better  
422 established. This latter observation is in accordance with the fact that, as receptacle  
423 compartments, aquatic environments are more susceptible to variations as further indicated  
424 by a high number of specific OTUs in the lake ( $3182 \pm 682$  OTUs) and river ( $1517 \pm 467$  OTUs)  
425 than in soil ( $1306 \pm 232$  OTUs).



426 Linear discriminant analysis effect size (LEfSe) revealed a difference in taxa abundance  
427 according to the compartments (soils, rivers and lakes) and seasons. LEfSe exhibited specific  
428 genera for each habitat that were more abundant ( $p < 0.05$ ) in soils than in both aquatic  
429 ecosystems (Fig. 4A). The habitat specificity was consistent with previous studies for  
430 microbial communities in coastal areas (Ben Salem et al., 2019; Jeanbille et al., 2016a; Xi et  
431 al., 2014) and soils (Chaudhary et al., 2015; Dong et al., 2019). Moreover, LEfSe showed a  
432 specificity of genera in each season/compartiment that were more frequent ( $p < 0.05$ ) in  
433 lakes habitats (Fig. 4B). The presence of particular genera, such as *Arenimonas*, *Bryobacter*  
434 and *Salinimicrobium*, that were found dominant ( $p < 0.05$ ) in lake sediments in winter, were  
435 also revealed dominant ( $p < 0.05$ ) in soil (*Arenimonas* in winter, and *Bryobacter* in summer)  
436 and in river (*Salinimicrobium* in summer) by LEfSe (Fig. 4B). Such observation also suggested  
437 the transfer of microorganisms from a compartment to another along the soil-river-lake  
438 continuum. Interestingly, several members of the *Arenimonas* genus have been isolated  
439 from ecosystems located at the soil/aquatic ecosystems interfaces, including sandy beaches  
440 (Kwon et al., 2007), estuaries (Jeong et al., 2016), and rice-fields (Zhang et al., 2015, 2017).  
441 Similarly, some members of the halophilic genus *Salinimicrobium* have been isolated from  
442 coastal sediments (Lee et al., 2012; Zhang et al., 2017), and from saline and reclaimed soils  
443 (Chen et al., 2008; Kim et al., 2016), while *Bryobacter* genus has been described mainly in  
444 soils and paddy soils (Kulichevskaya et al., 2010; Li et al., 2021). Thus, our study provides  
445 compelling evidence on the ecophysiology and ability of these taxa in colonizing and thriving  
446 within the mixing zones the soil-river-lake continuums according to the season.

447

#### 448 *Impact of pollutant on microbial communities*

449 The cluster correlation (ClusCor) analysis revealed the correlations between the abundance  
450 of individual prokaryotic genera and the concentrations of environmental pollutants (Fig. 5).  
451 The analysis revealed four main groups. The Group G1, showing strong positive correlations  
452 ( $0.5 < \text{Pearson correlation} < 0.8$ ,  $p < 0.05$ ) with Hg, As, Zn, Pb and the sum of metal  
453 concentrations, included *Nitrospira*, *Arenimonas*, *Dinghuibacter*, *Altererythrobacter*,  
454 *Microbacterium*, *Flavisolibacter*, and *Agromyces* genera (Fig. 5). Interestingly, the LEfSe  
455 analysis showed that these genera were found significantly more abundant ( $p < 0.05$ )  
456 according to the compartment and/or the season (Fig. 4) suggesting that they play a crucial

457 role as specialists in metal contaminated environments. Consistently, members related to  
458 these genera have been found predominant in metal contaminated environments, as for  
459 example *Microbacterium*, *Agromyces*, and *Dinghuibacter* in soils (Corretto et al., 2017, 2020;  
460 Lv et al., 2016; Xiao et al., 2019), *Nitrospira* and *Flavisolibacter* in soil and freshwater  
461 ecosystems (Das et al., 2016; Hong et al., 2015; Liu et al., 2018), and *Arenimonas* and  
462 *Altererythrobacter* in marine sediments (Jeong et al., 2013; Matsumoto et al., 2011). Thus,  
463 our results show that various taxa able to cope with metals are present in the different  
464 habitats along the soil-river-lake continuums, and there are most of the time metal and  
465 habitat specific.

466 The Group G2, including *Planctomyces*, *Variibacter*, *Streptomyces* and *Pir4lineage* genera,  
467 showed strong significant (Pearson correlation  $< -0.5$ ,  $p < 0.05$ ) negative correlations,  
468 particularly with the Pi (Fig. 5), suggesting that they are sensitive to the presence of multi-  
469 contaminants. Nevertheless, *Streptomyces* and *Planctomyces* genera showed positive  
470 correlations ( $0.5 < \text{Pearson correlation} < 0.6$ ,  $p < 0.05$ ) with petroleum hydrocarbon  
471 compounds (alkanes and PAHs), explaining that these genera were detected in petroleum  
472 hydrocarbon contaminated sites (Bachoon et al., 2001; Duran et al., 2015a). Additionally,  
473 LEfSe revealed that these genera were specifically more abundant ( $p < 0.05$ ) according to the  
474 compartment: *Streptomyces* for soil, *Variibacter* for river sediment, and for lake sediment  
475 *Planctomyces* in winter and *Pir4lineage* in summer (Fig. 4), further substantiating that these  
476 genera occupy specific niches (Dedysh et al., 2020; Nicault et al., 2020; Steven et al., 2011;  
477 Zhang et al., 2020). Such results suggested that these genera are probably sensitive not only  
478 to the presence of pollutant but also to the fluctuation of environmental parameters as they  
479 show habitat specificity.

480 The Group G3 gathered genera which abundance correlated with the presence of organic  
481 compounds (Fig. 5). Considering the strongest positive correlation (Pearson correlation  $> 0.6$ ,  
482  $p < 0.05$ ), three subgroups were revealed according to the nature of the main correlated  
483 compound. The "pesticide subgroup", correlated with OCPs, included *Rubroacter*, *Gaiella*,  
484 *Gemmata*, and *Microvirga* genera, which were shown significantly more abundant ( $p < 0.05$ )  
485 in the soil compartment by LEfSe (Fig. 4A), together with *Skermanella* and *Blastococcus*  
486 genera (Fig. 5). Such observation was consistent with previous reports indicating that  
487 members of these genera were found to be predominant in pesticide treated soils (Kumar et

488 al., 2020; Liu et al., 2020; Thelusmond et al., 2016), where they play important functional  
489 roles related to the nitrogen biogeochemical cycle (Aguiar et al., 2020; Yang et al., 2019).  
490 These genera were also correlated (Pearson correlation  $> 0.5$ ,  $p < 0.05$ ) with Cd and Cu (Fig.  
491 5), metals introduced in agricultural soils by the use of Cd-rich phosphate fertilizers (Li et al.,  
492 2020b) and copper antimicrobial compounds (Lamichhane et al., 2018). They have also been  
493 described in metal-contaminated soils (Liu et al., 2019a; Liu et al., 2019b; Liu et al., 2021). It  
494 is likely that the genera of the G3 "pesticide subgroup" own metabolic properties well  
495 adapted to the presence of agricultural contaminants such as OCPs and metals. *Massilia*  
496 genera and genera related to *Blastocatellaceae* RB41, that were shown significantly more  
497 abundant in the soil compartment by LEfSe ( $p < 0.05$ ), correlated (Pearson correlation  $> 0.8$ ,  
498  $p < 0.01$ ) with PAHs constituting the "PAH subgroup" (Fig. 5). Members of these genera are  
499 known for their capacities to degrade complex organic compounds (Huber et al., 2014;  
500 Pascual et al., 2015), including PAHs (Araujo et al., 2020; Bodour et al., 2003; Duran et al.,  
501 2015a). Finally, the *Acidibacter* genus formed the "alkane subgroup", also significantly found  
502 to be more abundant ( $p < 0.05$ ) in the soil compartment (LEfSe, Fig. 4A), consistently with  
503 the fact that members of this genus have been detected in oil-contaminated soils (He et al.,  
504 2020). Noteworthy, the genera constituting the three subgroups of Group G3 were found to  
505 be predominant in the soil compartment (LEfSe,  $p < 0.05$ , Fig. 4A) suggesting that these  
506 genera might play a pivotal role to cope with the presence of organic compounds in soils.  
507 Beside these three subgroups, Group G3 included genera showing significant positive  
508 correlation (Pearson correlation  $> 0.5$ ,  $p < 0.05$ ) with "alkanes" (*Pirellula*) and "alkanes +  
509 PAHs" (*Opitutus*, *Fusibacter*, and *Lysobacter*) or "OCPs" (*Nocardioides*, *Gemmatimonas*, and  
510 *Bryobacter*) and "OCPs + PAHs" (*Adhaeribacter* and *Solirubrobacter*) (Fig. 5). Interestingly,  
511 LEfSe showed that the genera correlated with petroleum hydrocarbons alone ("alkanes" and  
512 "alkanes + PAHs") were predominant in river sediment ( $p < 0.05$ ) while most genera  
513 correlated with pesticides and petroleum hydrocarbons ("OCPs" and "OCPs + PAHs") were  
514 predominant in soil ( $p < 0.05$ , Fig. 4). Such observation was in accordance with the  
515 contamination levels in the different compartments (Table 1). It is likely that these  
516 "specialist" bacteria, able to cope with the presence of contaminant type, are habitat (soil,  
517 river, and lake compartments) dependent. Such habitat specificity, described in previous  
518 studies (Ben Salem et al., 2017; Guo et al., 2016; Jeanbille et al., 2016a,b; Smii et al., 2015), is  
519 highlighted in our study. It was particularly noticeable for *Pirellula*, *Fusibacter*, *Nocardioides*,

520 and *Lysobacter* genera for river sediment, while *Adhaeribacter* and *Solirubrobacter* were  
521 specifically linked with soil (LEfSe,  $p < 0.05$ , Fig. 4). These genera have been proposed as  
522 pollution indicators: *Pirellula*, *Fusibacter*, and *Nocardioides* genera for freshwater sediments  
523 (Chen et al., 2016; Guo et al., 2016), while *Lysobacter*, *Adhaeribacter* and *Solirubrobacter*  
524 genera for chlorinated pesticide polluted soils (Aguiar et al., 2020; Cheng et al., 2020;  
525 Ramírez et al., 2020). Thus, despite the connection between compartments, our results  
526 indicated that these genera remained habitat specific. In contrast, *Gemmatimonas* and  
527 *Bryobacter* genera correlated with the presence of OCPs irrespective of the habitat, as they  
528 were found predominant in soil and lake sediment compartments (Fig. 4). These genera are  
529 described cosmopolitan being found in diverse aquatic and terrestrial ecosystems (Li et al.,  
530 2021; Zeng et al., 2016). The presence of these genera, able to inhabit various ecosystems, in  
531 the different compartments supports the connection between compartments within the  
532 soil-river-lake continuum, suggesting that they can be transferred from a compartment to  
533 another via running waters and runoff (Luo et al., 2020), which are important in winter in the  
534 Ichkeul Lake area (Ben Salem et al., 2016a).

535 Among genera included in the Group G4, three main subgroups were noticeable considering  
536 the strongest correlations: i) *Actibacter*, *Anaerolinea* and *Thiobacillus* genera showing  
537 significant positive correlations (Pearson correlation  $> 0.7$ ,  $p < 0.01$ ) with mercury content.  
538 *Actibacter* was found significantly abundant in aquatic sediments, *Thiobacillus* in the river  
539 and soil compartments (LEfSe,  $p < 0.05$ ), while LEfSe revealed no specific habitat for  
540 *Anaerolinea* (Fig. 4). Interestingly, *Anaerolinea* has been detected in mercury contaminated  
541 soil/water transition zone (Du et al., 2020), being more abundant in sediment than in soil. It  
542 is likely that this genus is well adapted to the variation of environmental parameters as that  
543 occurring in mixing zone such as soil-river-lake continuum. The metabolic capacities allowing  
544 *Anaerolinea* to occupy diverse habitats with the presence of mercury deserve to be further  
545 investigated to elucidate its ecological role. Among these three genera only *Thiobacillus* has  
546 been described able to transform mercury species (Huang et al., 2020; Vázquez-Rodríguez et  
547 al., 2015). *Thiobacillus* carry *mer* genes operon on either chromosome or plasmid (Velasco et  
548 al., 1999), the later allowing the spreading of *mer* genes within the microbial community by  
549 horizontal gene transfer (Mindlin et al., 2002). Horizontal gene transfer has been recognized  
550 as important adaptation mechanism providing “immunization” against pollutants to

551 microbial community (Poursat et al., 2019), which advocate functional gene analyses for the  
552 development of microbial bioindicators of pollution. Noteworthy, the abundance of these  
553 genera showed negative correlation (Pearson correlation  $< -0.5$ ,  $p < 0.05$ ) with organic  
554 compounds (OCPs and PAHs) content, consistent with the fact that *Anaerolinea* has been  
555 described sensitive to the presence of high molecular weight PAHs (Júlio et al., 2019). Such  
556 exclusion to organic compounds further confirmed these genera as mercury “specialists” .  
557 ii) *Pontibacter*, *Nafulsella*, *Salinimicrobium*, *Cesiribacter* showing significant positive  
558 correlation (Pearson correlation  $> 0.5$ ,  $p < 0.05$ ) with Ni and Cr, and significant negative  
559 correlation (Pearson correlation  $< -0.05$ ,  $p < 0.05$ ) with PAHs (Fig. 5). Such observation  
560 suggested that these four genera are probably “specialists” well adapted to the presence of  
561 Ni and Cr as they excluded with other pollutants. The LEfSe analysis (Fig. 4) revealed no  
562 specific habitat for the *Nafulsella* genera, while *Pontibacter*, *Salinimicrobium*, *Cesiribacter*  
563 were found to be dominant in the aquatic compartments (lake and/or river,  $p < 0.05$ ).  
564 Although information on these genera is scarce regarding metallic contaminants, the fact  
565 that *Pontibacter* has been detected in Cr-polluted soils (García-Gonzalo et al., 2017)  
566 reinforce our observation. iii) the *Bacillus* genus showing strong positive correlation (Pearson  
567 correlation  $> 0.5$ ,  $p < 0.05$ ) with PAHs, revealed predominant in lake sediment by LEfSe ( $p <$   
568  $0.05$ , Fig. 4A). It is likely that this genus has an important role in the Ichkeul Lake/Bizerte  
569 Lagoon complex as it has been detected in several sampling campaigns (Ben Said et al.,  
570 2008; Louati et al., 2013).

571

572 The ClusCor analysis showed four main groups of genera (G1-4) showing either co-  
573 occurrence or exclusion with pollutant in the different compartments, which represent  
574 potential bio-indicators for assessing ecosystem health as previously proposed (Lladó et al.,  
575 2018; Savvaidis et al., 2001; Segata et al., 2011). This information is useful for establishing  
576 ecological status of an ecosystem by including the bio-indicator genera in databases used for  
577 calculating microbial indices such as microgAMBI (Aylagas et al., 2017; Borja 2018). The  
578 observed links between the abundance of specific genera and contaminants suggested that  
579 identified genera might have metabolic capacities conferring a specific role in pollutant  
580 transformation or degradation and ecosystem functioning that, in turn, influence the overall  
581 microbial community assemblage, which also depends on environmental parameters such as  
582 pH, nutrients and oxygen availability. In order to unveil the specific functional role of the

583 four groups of genera, their functional capabilities were inferred by PRICRUST2 analysis  
584 focusing on specific functions related to the presence of pollutants, including hydrocarbon  
585 and pesticide degradation, and metal transformation or resistance. The nearest sequenced  
586 taxon indices (NSTI) ranged between 0.09 and 0.29 indicating the reliability of the PICRUST2  
587 prediction analysis. The abundance distribution (supplementary material, Fig. S4) and the  
588 comparison of the identified genera groups, based on their predicted functional capabilities,  
589 revealed that they shared most of the functions related to pollutants (Fig. 6). Consistently  
590 with the ClusCor analysis (Fig. 5) the groups G1 and G3 were specifically linked with genes  
591 involved in metal transformation/resistance, as for example: K19591 and K19592 (Hg and Cu  
592 resistance), K08364 and K08365 (Hg resistance), and K015726 and K015727 (Co, Zn and Cd  
593 resistance) for Group G1, while Group G3 was linked with As (K11811), Zn (K13638), and Hg  
594 (K08363 and K00520) resistance genes (supplementary material, Table S3). The differences  
595 on metal transformation/resistance genes between Group G1 and Group G3 probably reflect  
596 the habitat specificity: members of the Group G1 being more cosmopolitan (found in the  
597 three compartments) than those of Group G3, which were particularly found to be more  
598 abundant in the soil compartment (Fig. 4).

599 All genera specifically exhibited genes involved in pesticide degradation, genera from Group  
600 G3 being linked with atrazine (K03383) and other pesticide degradation (K13992) genes.  
601 Noteworthy, the Group G3 was specifically linked with several genes (K03391, K14419,  
602 K14028, K14029, K04098, and K01561) involved in chlorinated and halogenated compounds  
603 degradation (supplementary material, Table S3). Such information suggested that these  
604 genes might be involved in chlorinated pesticides (OCPs) degradation as shown for the  
605 pentachlorophenol monooxygenase gene (K03391) from *Sphingobium chlorophenolicum*  
606 (Flood and Copley 2018). It is likely that the functional capacities involved in OCPs  
607 degradation represent an asset for soil bacteria to thrive in the presence of xenobiotics since  
608 members of Group G3 were found dominant in soil by the LefSe analysis (Fig. 4).

609 Interestingly, the groups G1 and G2 were linked with genes connected with the degradation  
610 of aromatic compounds. The Group G1 being associated with di- and mono-oxygenase  
611 (K04100, K04101, and K03380) genes, whilst the Group G2 was linked with genes involved in  
612 PAH degradation including dehydrogenase (K13954), hydroxylase (K00480), decarboxylase  
613 (K04102), and CytP450 (K14338) genes, which was consistent with the ClusCor analysis

614 showing the correlation of Group G2 members with PAH content (Fig. 5). As observed for  
615 metals, the differences shown by Group G1 and Group G2 regarding PAH degradation genes  
616 might reflect the habitat specificity as members of the Group G2 were specifically found to  
617 be more abundant in the aquatic compartments (Fig. 4).

618 The Group G4 was linked with genes contributing to anaerobic metabolism such sulfate  
619 reduction (K11181) and nitrogen fixation (K02586, K02588, and K02591), together with  
620 genes participating to the aerobic degradation of aromatic compounds (supplementary  
621 material, Table S3), including CytP450 (K01253), dehydrogenase (K10217), and dioxygenase  
622 (K05784) genes. The fact that Group G4 encompassed anaerobic and aerobic metabolisms  
623 was consistent with the LefSe analysis showing that Group G4 included genera with specific  
624 habitat (soil, river or lake compartment) together with genera without habitat specificity  
625 (Fig. 4).

626 Thus, the PICRUSt2 functional prediction was consistent with the ClusCor analysis, indicating  
627 that each identified genera group displays functions specifically related to a type of pollutant  
628 that correspond to "specialists" taxa as proposed in previously studies reporting bacterial  
629 populations specifically associated with the presence of pollutants (Ben Salem et al., 2019;  
630 Duran et al., 2015a; Lladó et al., 2018). It is likely that the different "specialists" cohabit in  
631 the environment participating to a metabolic network that organize the functional capacities  
632 in response to the presence of multi-contaminants according to the habitat specificity, as  
633 observed in mine-polluted sites (Liu et al., 2018, 2019) and marine polluted sediments  
634 (Duran et al., 2015b; McGenity 2014). Further studies are required to understand the  
635 parameter, including physical-chemical factors, shaping the microbial and metabolic  
636 network organization.

637

638 In this study, the comparison of microbial community composition along the different  
639 transects of the soil-river-lake continuums revealed a relatively large number of shared OTUs  
640 along the soil-river-lake continuum. Such observation indicated bacterial community  
641 coalescence where the soil microbes, and the suspended matter entering the aquatic  
642 ecosystem through runoff are mixed with the microbial population inhabiting river and lake  
643 sediments. Moreover, the study revealed that the organisation of microbial communities

644 was shaped by the presence of pollutant and by habitat filtering. Microbial genera groups  
645 were identified as "specialist" taxa exhibiting key degradation/transformation functions  
646 involved in metabolisms activated in response to the presence of pollutants. The study  
647 provides novel information on microbial community organisation in soil-river-lake  
648 continuums submitted to multi-contamination. The results obtained in this study pave the  
649 way for future investigations to better understand the organisation and functioning of  
650 microbial communities in soil-river-lake continuum mixing zone, where microorganisms and  
651 organic materials are transferred from a compartment to another. The expected information  
652 will be of paramount importance for the development of bio-indicators reporting on the  
653 recovery capacities of a contaminated ecosystem. Several studies propose microbial  
654 biomarker of contamination (Ben Salem et al., 2019; Ford et al., 2005; Ventrino et al.,  
655 2018), particularly integrative microbial indices such as microgAMBI (Aylagas et al., 2017;  
656 Borja 2018) that in our study is consistent with the chemical pressure index. Including  
657 specialist microorganisms and considering also their metabolic capacities in such integrative  
658 indices would help to develop accurate biomarker of contamination (Ventrino et al., 2018).  
659 However, several environmental factors (e.g. pH, nutrients and oxygen availability) affect the  
660 microbial community composition and structure (Duran et al. 2015a,b), which have to be  
661 considered when developing biomarkers.

662

663

664

#### 665 **Author contributions**

666 **Zeina Bourhane:** Conceptualisation, Investigation, Resources, Formal analysis, Writing –  
667 original draft. **Anders Lanzen:** Investigation, Formal analysis, Validation, Writing. **Christine**  
668 **Cagnon:** Conceptualisation, Formal analysis, Validation, Supervision, Writing – original draft.  
669 **Olfa Ben Said:** Conceptualisation, Validation. **Ezzeddine Mahmoudi:** Validation. **Frederic**  
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675

676 **Declaration of competing interest**

677 The authors declare that they have no known competing financial interest or personal  
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679

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1137

1138 **Figure caption**

1139 **Fig. 1. Localisation of the sampling stations S2, S3 and S6 located around the Ichkeul Lake**  
1140 **(Tunisia).** At each station a continuum consisting of agricultural soil, river sediment, and lake  
1141 sediments was sampled, the distances between the compartments at each station are  
1142 indicated. O. Oued (wadi) followed by the river name.

1143

1144 **Fig. 2. Microbial community compositions at the phylum level along the continuum sites**  
1145 **(soil, river and lake) at each station (S2, S3 and S6) in summer (S) and winter (W).** The bar  
1146 plots represent the mean of three replicates. Other, phyla with relative abundance < 5%.

1147

1148 **Fig. 3. Comparison of microbial communities inhabiting the continuum (soil, river and lake)**  
1149 **at each station (S2, S3 and S6) in summer and winter.** (A) non-metric multidimensional  
1150 scaling (NMDS). The arrow represents the significant environmental variable ( $\Sigma$  PAHs;  $p <$   
1151  $0.05$ ) fitted as a vector using the envfit function. 2D stress: 0.095. (B) PERMANOVA  
1152 partitioning ( $R^2$  value,  $p$ -value < 0.001) according to compartments, stations, seasons and  
1153 their interactions.

1154

1155 **Fig. 4. Comparison of microbial communities from the stations located around the Ichkeul**  
1156 **Lake.** Linear discriminant analysis effect size (LEfSe) comparing (A) the compartments (soil,  
1157 river and lake) and (B) the season (summer and winter) within each compartment.

1158

1159 **Fig. 5. Correlation between microbial OTUs and pollutant concentrations by clustered**  
1160 **correlation (ClusCor) analysis.** The analysis is based on Pearson correlation, red indicating  
1161 high positive correlation and blue high negative correlation ( $p$  value < 0.01). Clustering was  
1162 performed applying Pearson correlations and Ward algorithm as cluster method. The  
1163 analysis was performed at the genus level applying a threshold similarity of 97% for OTU  
1164 identification. The specific OTUs revealed by LEfSe analysis are coloured according to their  
1165 specificity according to compartment: soil (blue circle), river (green triangle) and lake (red

1166 square); or the season: summer (full symbol) and winter (empty symbol).  $\Sigma$  Alkanes, sum of  
1167 alkane compounds;  $\Sigma$  PAHs, sum of polyaromatic hydrocarbons;  $\Sigma$  OCPs, sum of  
1168 organochlorine pesticides;  $\Sigma$  metals, sum of metals; Pi, pressure index.

1169

1170 **Fig. 6. Comparison of PICRUSt-predicted pollutant degradation/transformation capabilities**

1171 **of the genera groups (G1-G4).** The genera groups (G1-G4) identified by the ClusCor analysis

1172 (Fig. 5) were compared by NMDS based on PICRUSt-predicted genes involved in pollutant

1173 degradation/transformation, including metals (red), pesticides (green), and hydrocarbons

1174 (black). The most relevant genes specifically abundant in each genera group discussed in the

1175 text are identified by numbers and their description provided in supplementary material

1176 Table S3.

1177

**Table 1: Chemical analyses of contaminants in the different compartments (soil, and river and lake sediments) located at the stations S2, S3, and S6 around the Ichkeul Lake, in summer (S) and winter (W).** The data corresponds to the sum of the different types of pollutants: PAH: Polycyclic Aromatic Hydrocarbons; OCP: organochlorine pesticides; FLA: Fluoranthene; PYR: Pyrene. Average  $\pm$  SD are presented (n = 3).

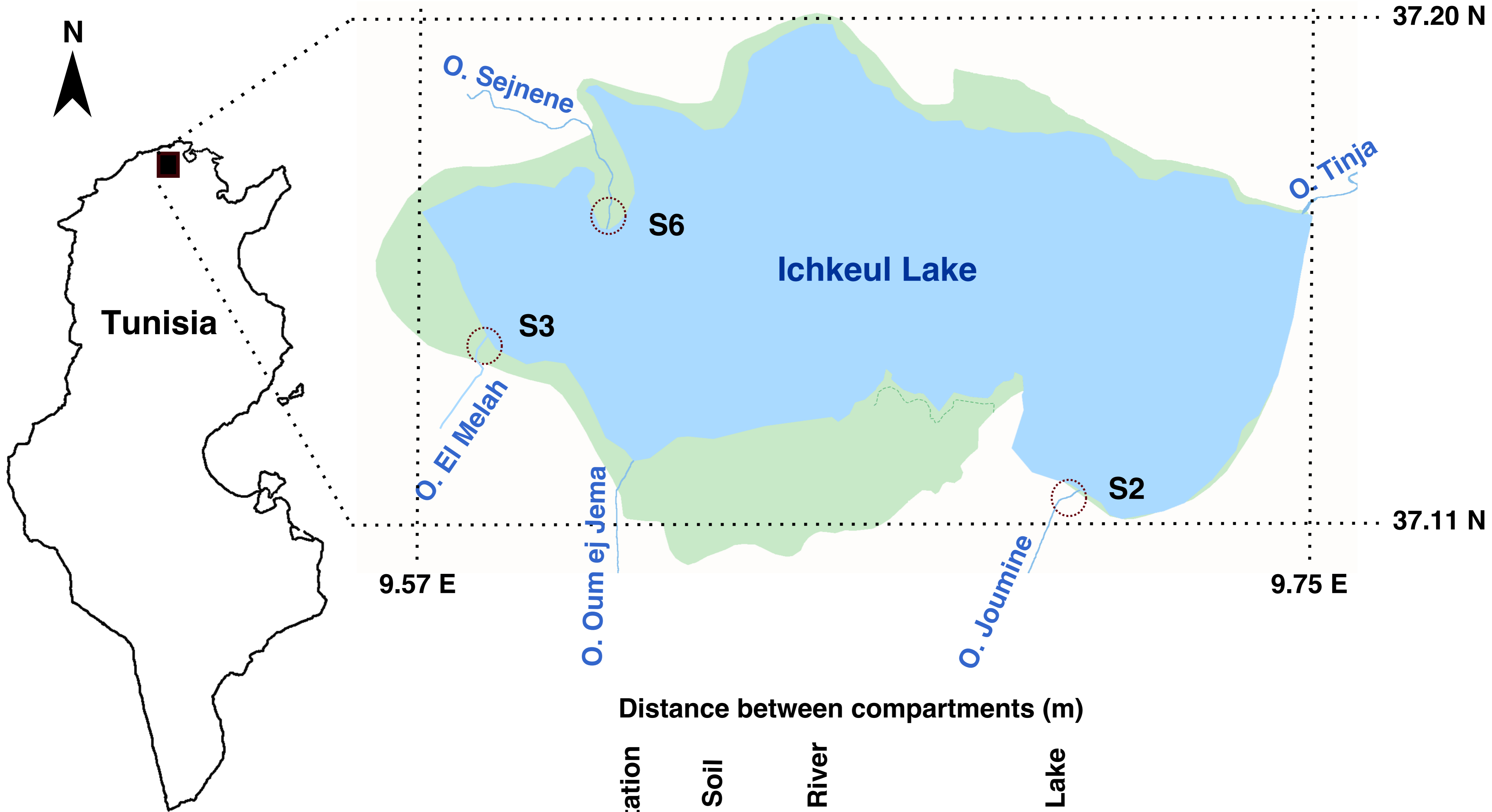
Pollutants	Stations										
	Compounds	Soil	S2			S3			S6		
			River	Lake	Soil	River	Lake	Soil	River	Lake	
$\Sigma$ Alkanes (ng g <sup>-1</sup> )	S	542 $\pm$ 16 <sup>b</sup>	368 $\pm$ 21 <sup>e</sup>	417 $\pm$ 16 <sup>d</sup>	193 $\pm$ 8 <sup>f</sup>	141 $\pm$ 7 <sup>g</sup>	140 $\pm$ 13 <sup>g</sup>	637 $\pm$ 10 <sup>a</sup>	472 $\pm$ 14 <sup>c</sup>	489 $\pm$ 12 <sup>c</sup>	
	W	1127 $\pm$ 63 <sup>a</sup>	545 $\pm$ 27 <sup>c</sup>	893 $\pm$ 38 <sup>b</sup>	301 $\pm$ 27 <sup>d</sup>	176 $\pm$ 11 <sup>e</sup>	234 $\pm$ 11 <sup>de</sup>	806 $\pm$ 26 <sup>b</sup>	572 $\pm$ 27 <sup>c</sup>	565 $\pm$ 21 <sup>c</sup>	
$\Sigma$ PAHs (ng g <sup>-1</sup> )	S	649 $\pm$ 8 <sup>d</sup>	288 $\pm$ 4 <sup>h</sup>	567 $\pm$ 6 <sup>e</sup>	481 $\pm$ 5 <sup>f</sup>	394 $\pm$ 3 <sup>g</sup>	409 $\pm$ 2 <sup>g</sup>	1210 $\pm$ 21 <sup>a</sup>	683 $\pm$ 3 <sup>c</sup>	905 $\pm$ 8 <sup>b</sup>	
	W	807 $\pm$ 10 <sup>c</sup>	322 $\pm$ 5 <sup>h</sup>	680 $\pm$ 8 <sup>d</sup>	617 $\pm$ 26 <sup>e</sup>	415 $\pm$ 13 <sup>g</sup>	504 $\pm$ 15 <sup>f</sup>	1436 $\pm$ 26 <sup>a</sup>	816 $\pm$ 5 <sup>c</sup>	973 $\pm$ 26	
$\Sigma$ OCPs (ng g <sup>-1</sup> )	S	233 $\pm$ 10 <sup>a</sup>	76 $\pm$ 7 <sup>ef</sup>	158 $\pm$ 4 <sup>b</sup>	172 $\pm$ 9 <sup>b</sup>	61 $\pm$ 2 <sup>f</sup>	124 $\pm$ 2 <sup>c</sup>	106 $\pm$ 2 <sup>d</sup>	42 $\pm$ 5 <sup>g</sup>	84 $\pm$ 2 <sup>e</sup>	
	W	214 $\pm$ 8 <sup>b</sup>	69 $\pm$ 7 <sup>f</sup>	157 $\pm$ 8 <sup>c</sup>	248 $\pm$ 4 <sup>a</sup>	65 $\pm$ 1 <sup>f</sup>	134 $\pm$ 4 <sup>d</sup>	105 $\pm$ 2 <sup>d</sup>	40 $\pm$ 1 <sup>g</sup>	71 $\pm$ 4 <sup>f</sup>	
$\Sigma$ Metals (mg Kg <sup>-1</sup> )	S	332 $\pm$ 27 <sup>a</sup>	816 $\pm$ 445 <sup>a</sup>	399 $\pm$ 6 <sup>a</sup>	635 $\pm$ 529 <sup>a</sup>	2421 $\pm$ 2458 <sup>a</sup>	1992 $\pm$ 1120 <sup>a</sup>	452 $\pm$ 155 <sup>a</sup>	300 $\pm$ 55 <sup>a</sup>	432 $\pm$ 176 <sup>a</sup>	
	W	431 $\pm$ 35 <sup>b</sup>	496 $\pm$ 72 <sup>b</sup>	473 $\pm$ 52 <sup>b</sup>	2536 $\pm$ 677 <sup>a</sup>	1206 $\pm$ 1265 <sup>ab</sup>	347 $\pm$ 95 <sup>a</sup>	218 $\pm$ 20 <sup>b</sup>	309 $\pm$ 38 <sup>a</sup>	323 $\pm$ 79 <sup>a</sup>	
FLA/(FLA + PYR)	S	0.44 $\pm$ 0.002 <sup>de</sup>	0.35 $\pm$ 0.01 <sup>e</sup>	0.40 $\pm$ 0.012 <sup>de</sup>	0.60 $\pm$ 0.007 <sup>abcd</sup>	0.54 $\pm$ 0.03 <sup>bcde</sup>	0.51 $\pm$ 0.01 <sup>cde</sup>	0.80 $\pm$ 0.04 <sup>a</sup>	0.78 $\pm$ 0.05 <sup>ab</sup>	0.73 $\pm$ 0.21 <sup>abc</sup>	
	W	0.44 $\pm$ 0.002 <sup>bc</sup>	0.35 $\pm$ 0.01 <sup>c</sup>	0.40 $\pm$ 0.012 <sup>bc</sup>	0.51 $\pm$ 0.013 <sup>abc</sup>	0.51 $\pm$ 0.01 <sup>abc</sup>	0.51 $\pm$ 0.01 <sup>abc</sup>	0.80 $\pm$ 0.04 <sup>a</sup>	0.66 $\pm$ 0.15 <sup>ab</sup>	0.73 $\pm$ 0.21 <sup>a</sup>	
Pressure index (Pi)	S	2.35 $\pm$ 0.1 <sup>ab</sup> (III)*	2.47 $\pm$ 0.3 <sup>ab</sup> (III)*	2.48 $\pm$ 0.1 <sup>ab</sup> (III)*	2.18 $\pm$ 0.3 <sup>ab</sup> (III)*	2.45 $\pm$ 0.4 <sup>ab</sup> (III)*	2.96 $\pm$ 0.1 <sup>a</sup> (III)*	2.18 $\pm$ 0.1 <sup>ab</sup> (III)*	1.78 $\pm$ 0.2 <sup>b</sup> (II)*	1.97 $\pm$ 0.4 <sup>b</sup> (II)*	
	W	2.51 $\pm$ 0.1 <sup>ab</sup> (III)*	2.49 $\pm$ 0.04 <sup>ab</sup> (III)*	2.55 $\pm$ 0.2 <sup>ab</sup> (III)*	3.15 $\pm$ 0.1 <sup>a</sup> (IV)*	2.57 $\pm$ 0.5 <sup>ab</sup> (III)*	2.07 $\pm$ 0.2 <sup>b</sup> (III)*	2.31 $\pm$ 0.04 <sup>b</sup> (III)*	2.38 $\pm$ 0.1 <sup>b</sup> (III)*	2.38 $\pm$ 0.4 <sup>b</sup> (III)*	

\*Classification according to Aylagas et al. (2017) determining the ecological status: I, very good; II, good; III, moderate; IV, bad. The same small letter indicates no significant difference of means compared by one-way ANOVA,  $p < 0.05$ .

**Table 2: Alpha diversity indices of microbial community from the compartments (soil, and river and lake sediments) located at the stations S2, S3, and S6, around the Ichkeul Lake in summer (S) and winter (W). Average  $\pm$  SD are presented (n = 3).**

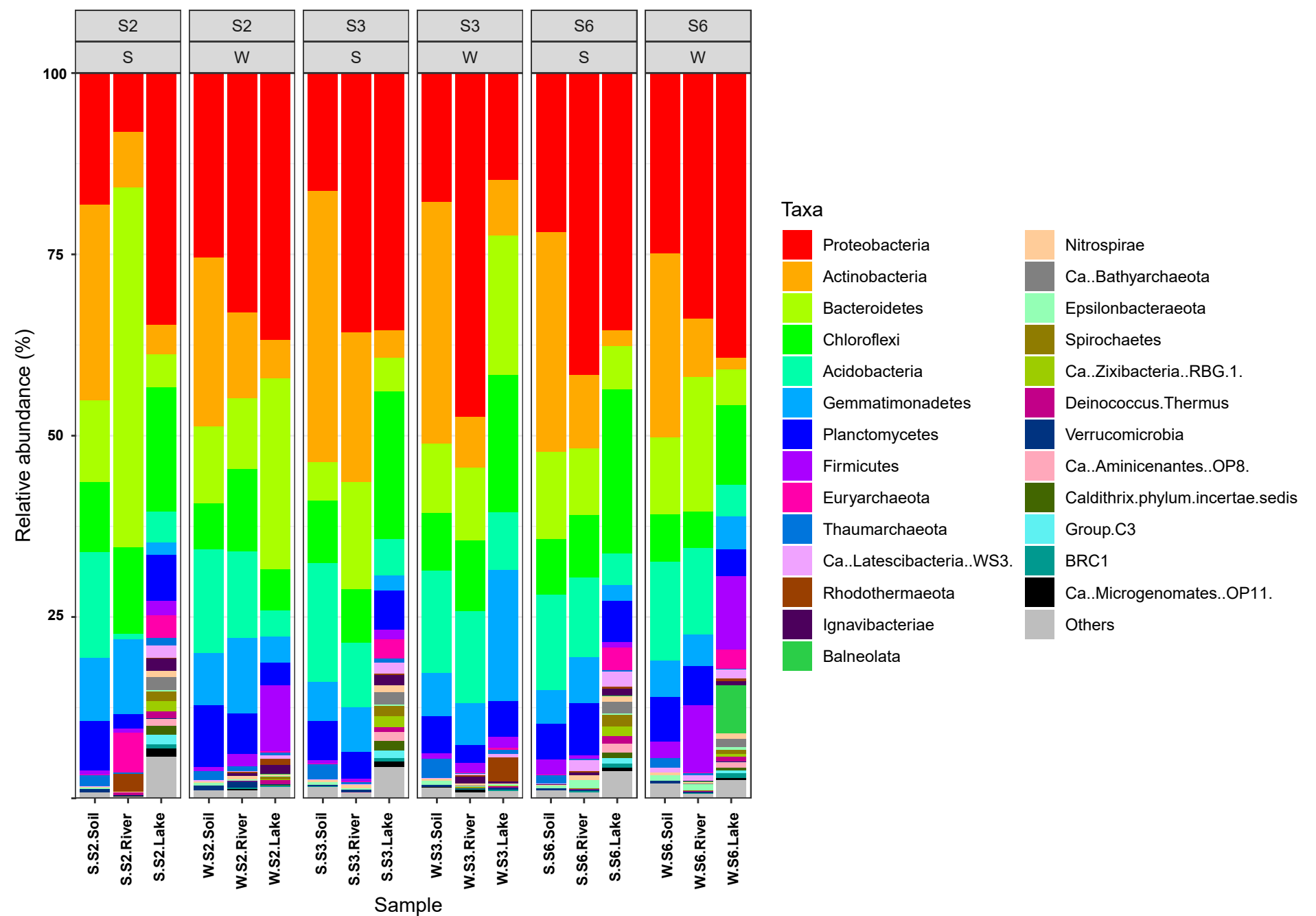
Indices	Stations									
	S2			S3			S6			
	Soil	River	Lake	Soil	River	Lake	Soil	River	Lake	
Reads	S	47303 $\pm$ 19965 <sup>a</sup>	33009 $\pm$ 9274 <sup>a</sup>	32831 $\pm$ 12617 <sup>a</sup>	38955 $\pm$ 7804 <sup>a</sup>	29565 $\pm$ 78004 <sup>a</sup>	35049 $\pm$	42102 $\pm$ 33195 <sup>a</sup>	34468 $\pm$ 90550 <sup>a</sup>	25586 $\pm$ 6204 <sup>a</sup>
	W	28232 $\pm$ 2847 <sup>a</sup>	28905 $\pm$ 3366 <sup>a</sup>	35516 $\pm$ 2599 <sup>a</sup>	56123 $\pm$ 20811 <sup>a</sup>	28519 $\pm$ 15676 <sup>a</sup>	20916 <sup>a</sup> 41037 $\pm$ 9756 <sup>a</sup>	40780 $\pm$ 13768 <sup>a</sup>	28532 $\pm$ 9685 <sup>a</sup>	34870 $\pm$ 5544 <sup>a</sup>
Trimmed sequences	S	14604 $\pm$ 5295 <sup>a</sup>	13834 $\pm$ 4175 <sup>a</sup>	11965 $\pm$ 5184 <sup>a</sup>	13357 $\pm$ 2046 <sup>a</sup>	9947 $\pm$ 679 <sup>a</sup>	12093 $\pm$ 6902 <sup>a</sup>	9805 $\pm$ 5825 <sup>a</sup>	10666 $\pm$ 2992 <sup>a</sup>	9263 $\pm$ 3087 <sup>a</sup>
	W	11960 $\pm$ 293 <sup>a</sup>	13154 $\pm$ 1426 <sup>a</sup>	14256 $\pm$ 1547 <sup>a</sup>	21009 $\pm$ 995 <sup>a</sup>	13687 $\pm$ 7745 <sup>a</sup>	17090 $\pm$ 3209 <sup>a</sup>	18928 $\pm$ 6628 <sup>a</sup>	10557 $\pm$ 3686 <sup>a</sup>	16989 $\pm$ 2394 <sup>a</sup>
Species richness ( <i>R</i> )	S	2329 $\pm$ 354 <sup>a</sup>	875 $\pm$ 143 <sup>b</sup>	2297 $\pm$ 553 <sup>a</sup>	2108 $\pm$ 182 <sup>ab</sup>	1948 $\pm$ 254 <sup>ab</sup>	2117 $\pm$ 713 <sup>ab</sup>	2046 $\pm$ 689 <sup>b</sup>	2534 $\pm$ 427 <sup>a</sup>	2012 $\pm$ 355 <sup>ab</sup>
	W	2331 $\pm$ 51 <sup>ab</sup>	2302 $\pm$ 136 <sup>b</sup>	2752 $\pm$ 578 <sup>ab</sup>	2982 $\pm$ 78 <sup>ab</sup>	1899 $\pm$ 605 <sup>b</sup>	1700 $\pm$ 270 <sup>b</sup>	3243 $\pm$ 538 <sup>a</sup>	2715 $\pm$ 693 <sup>ab</sup>	2325 $\pm$ 134 <sup>ab</sup>
Simpson ( <i>1-D</i> )	S	0.99 $\pm$ 0.0005 <sup>a</sup>	0.96 $\pm$ 0.006 <sup>b</sup>	0.99 $\pm$ 0.001 <sup>a</sup>	0.99 $\pm$ 0.0003 <sup>a</sup>	0.99 $\pm$ 0.002 <sup>a</sup>	0.99 $\pm$ 0.001 <sup>a</sup>	0.99 $\pm$ 0.001 <sup>a</sup>	0.99 $\pm$ 0.001 <sup>a</sup>	0.99 $\pm$ 0.0003 <sup>a</sup>
	W	0.99 $\pm$ 0.002 <sup>a</sup>	0.99 $\pm$ 0.003 <sup>abc</sup>	0.99 $\pm$ 0.001 <sup>abc</sup>	0.98 $\pm$ 0.001 <sup>a</sup>	0.99 $\pm$ 0.003 <sup>d</sup>	0.99 $\pm$ 0.0001 <sup>bcd</sup>	0.99 $\pm$ 0.002 <sup>a</sup>	0.99 $\pm$ 0.001 <sup>a</sup>	0.99 $\pm$ 0.0008 <sup>a</sup>
Shannon ( <i>H</i> )	S	6.67 $\pm$ 0.1 <sup>ab</sup>	4.66 $\pm$ 0.18 <sup>c</sup>	6.47 $\pm$ 0.23 <sup>ab</sup>	6.47 $\pm$ 0.02 <sup>ab</sup>	6.55 $\pm$ 0.27 <sup>ab</sup>	6.32 $\pm$ 0.15 <sup>b</sup>	6.56 $\pm$ 0.15 <sup>ab</sup>	6.90 $\pm$ 0.16 <sup>a</sup>	6.51 $\pm$ 0.08 <sup>ab</sup>
	W	6.77 $\pm$ 0.03 <sup>a</sup>	6.55 $\pm$ 0.06 <sup>a</sup>	6.49 $\pm$ 0.32 <sup>ab</sup>	6.78 $\pm$ 0.10 <sup>a</sup>	5.97 $\pm$ 0.07 <sup>c</sup>	6.01 $\pm$ 0.07 <sup>c</sup>	6.94 $\pm$ 0.1 <sup>a</sup>	6.89 $\pm$ 0.28 <sup>a</sup>	6.07 $\pm$ 0.03 <sup>bc</sup>
microAMBI*	S	-	3.13 $\pm$ 0.2 <sup>a</sup> (M)*	2.01 $\pm$ 0.1 <sup>b</sup> (G)*	-	1.69 $\pm$ 0.01 <sup>bc</sup> (G)*	1.97 $\pm$ 0.2 <sup>b</sup> (G)*	-	1.45 $\pm$ 0.2 <sup>c</sup> (G)*	1.97 $\pm$ 0.04 <sup>b</sup> (G)*
	W	-	1.45 $\pm$ 0.3 <sup>a</sup> (G)*	3 $\pm$ 0.02 <sup>b</sup> (M)*	-	1.62 $\pm$ 0.08 <sup>b</sup> (G)*	1.58 $\pm$ 0.4 <sup>b</sup> (G)*	-	1.63 $\pm$ 0.5 <sup>b</sup> (G)*	1.88 $\pm$ 0.01 <sup>b</sup> (G)*

\*microAMBI was not calculated for soil sites; It can be applied only for sediments either from river or lake. Classification according to Aylagas et al. (2017) determining the ecological status: G, good; M, moderate. The same small letter indicates no significant difference of means compared by one-way ANOVA,  $p < 0.05$ .

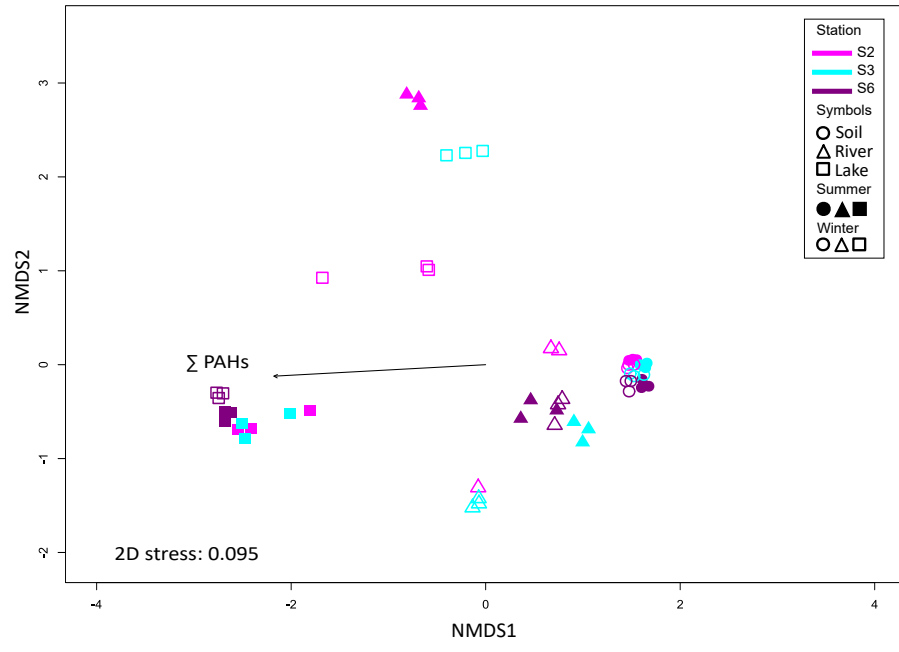
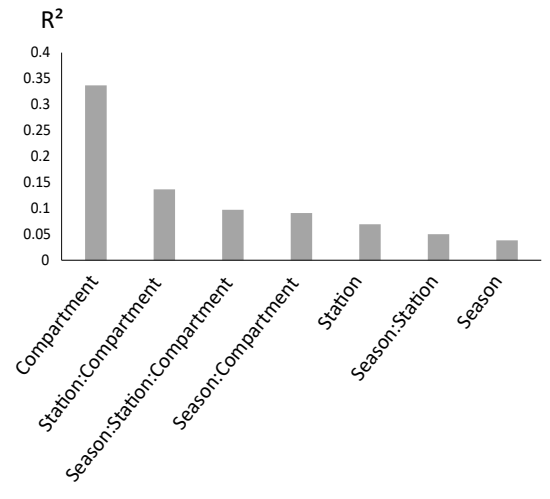


**Distance between compartments (m)**

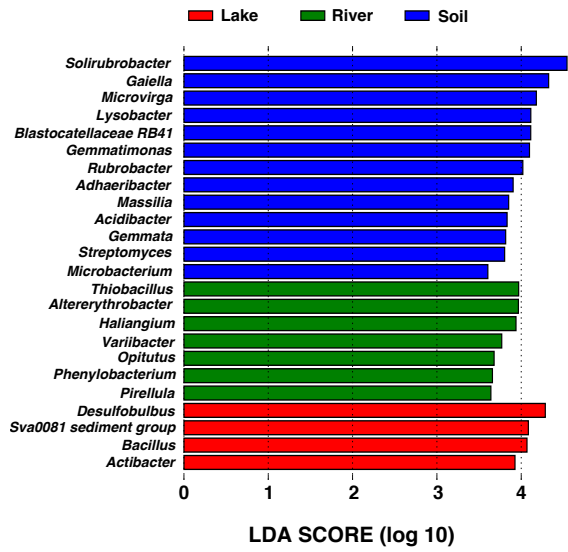
Station	Soil	River	Lake
S2	300 - 800	300 - 800	
S3	50 - 200	1800 - 2200	
S6	50 - 200	1000 - 1500	



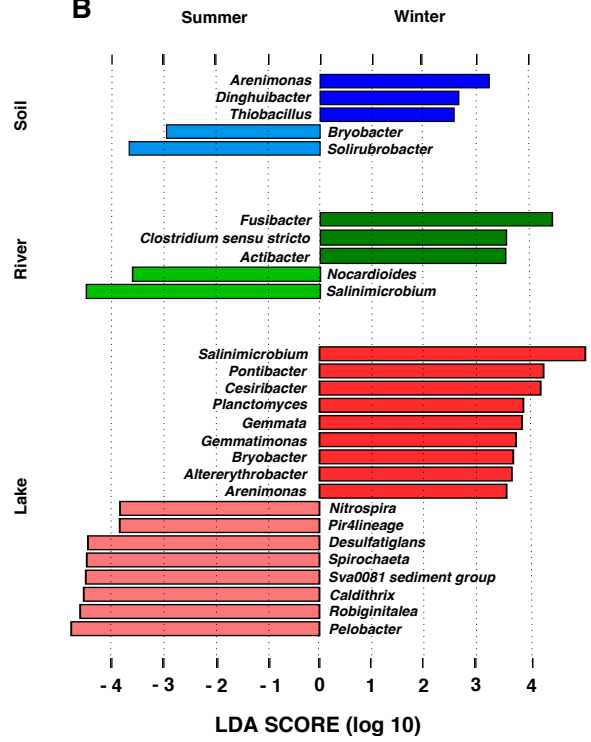


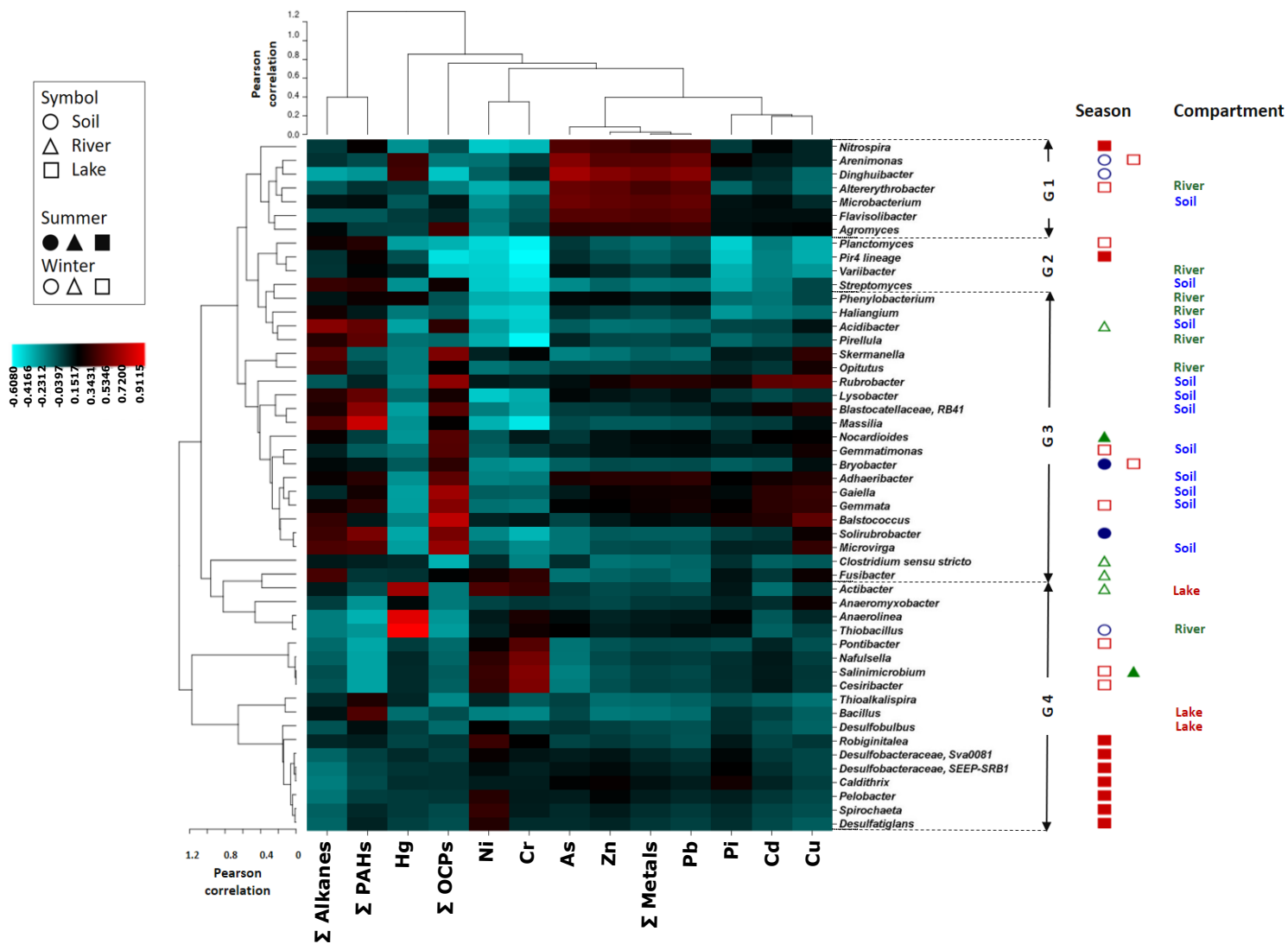
**A****B**

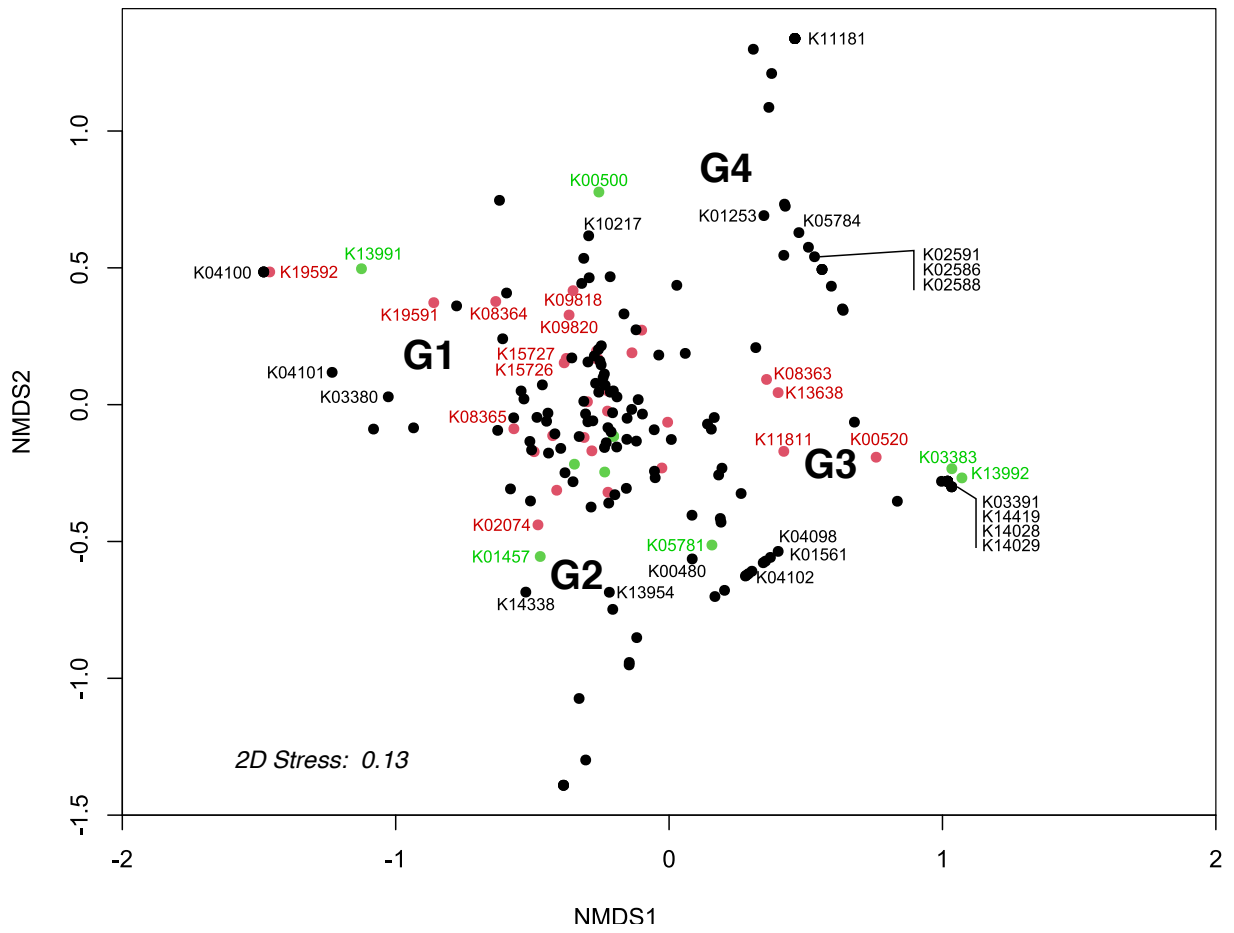
**A**

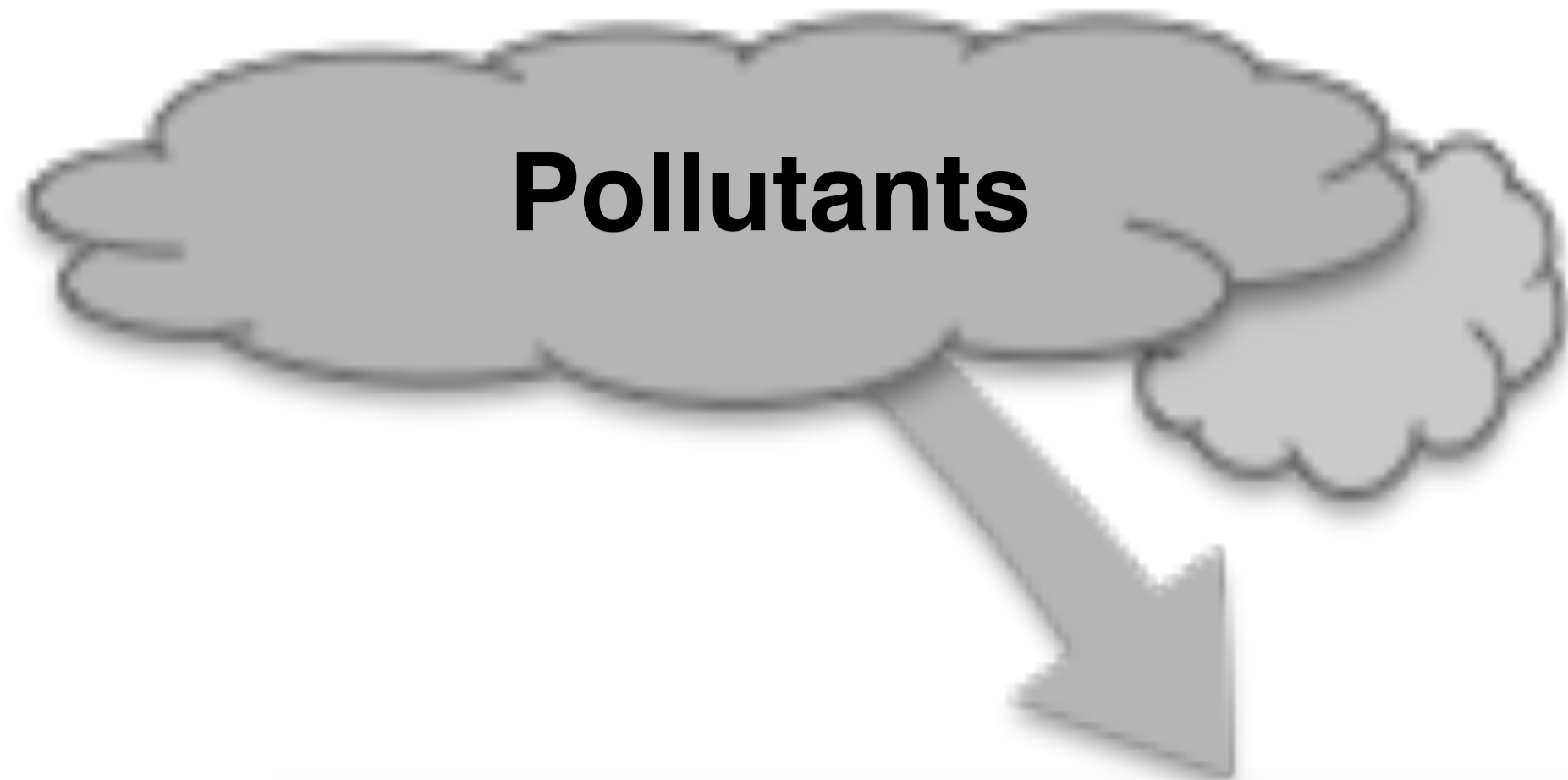


**B**

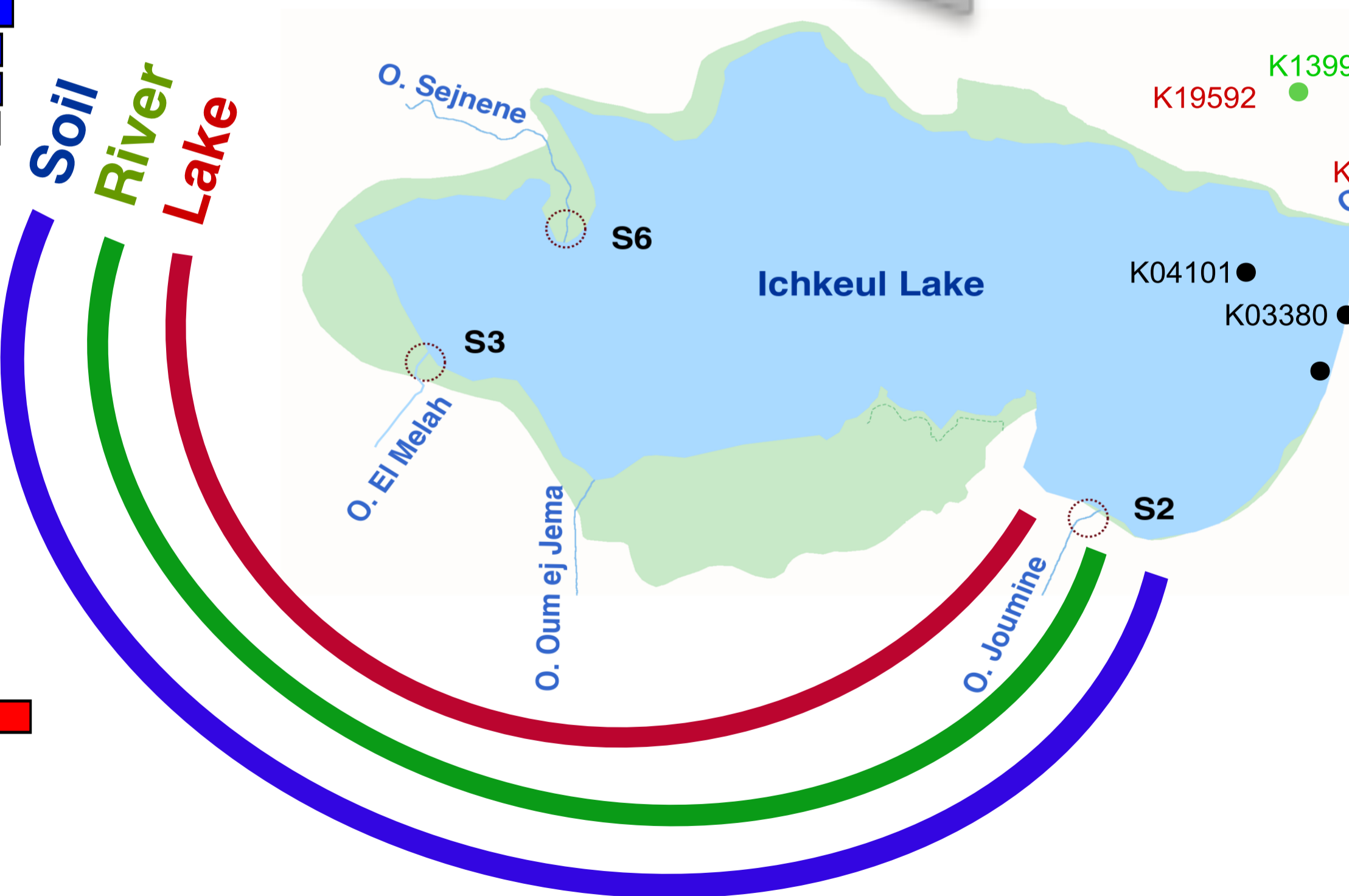
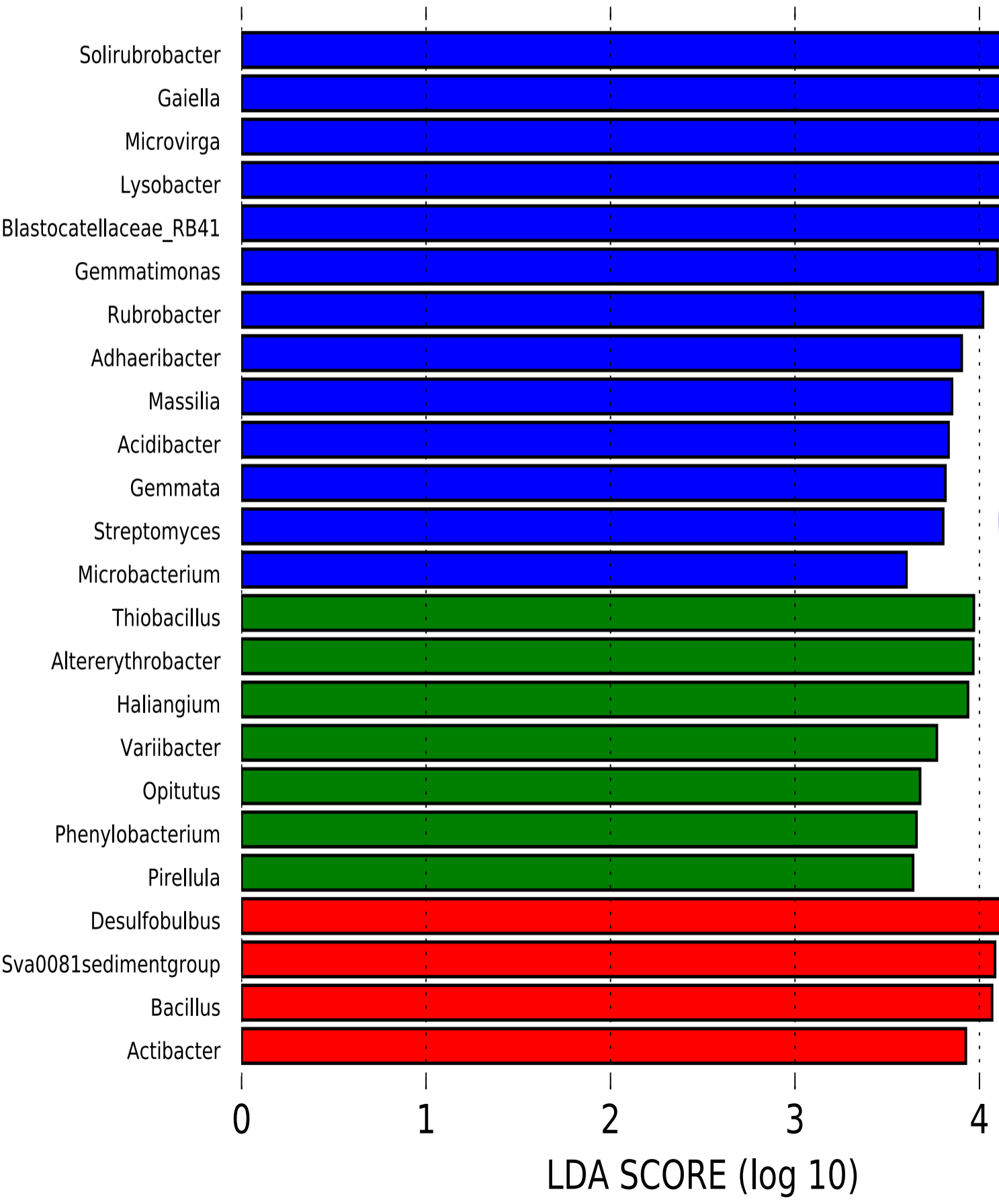




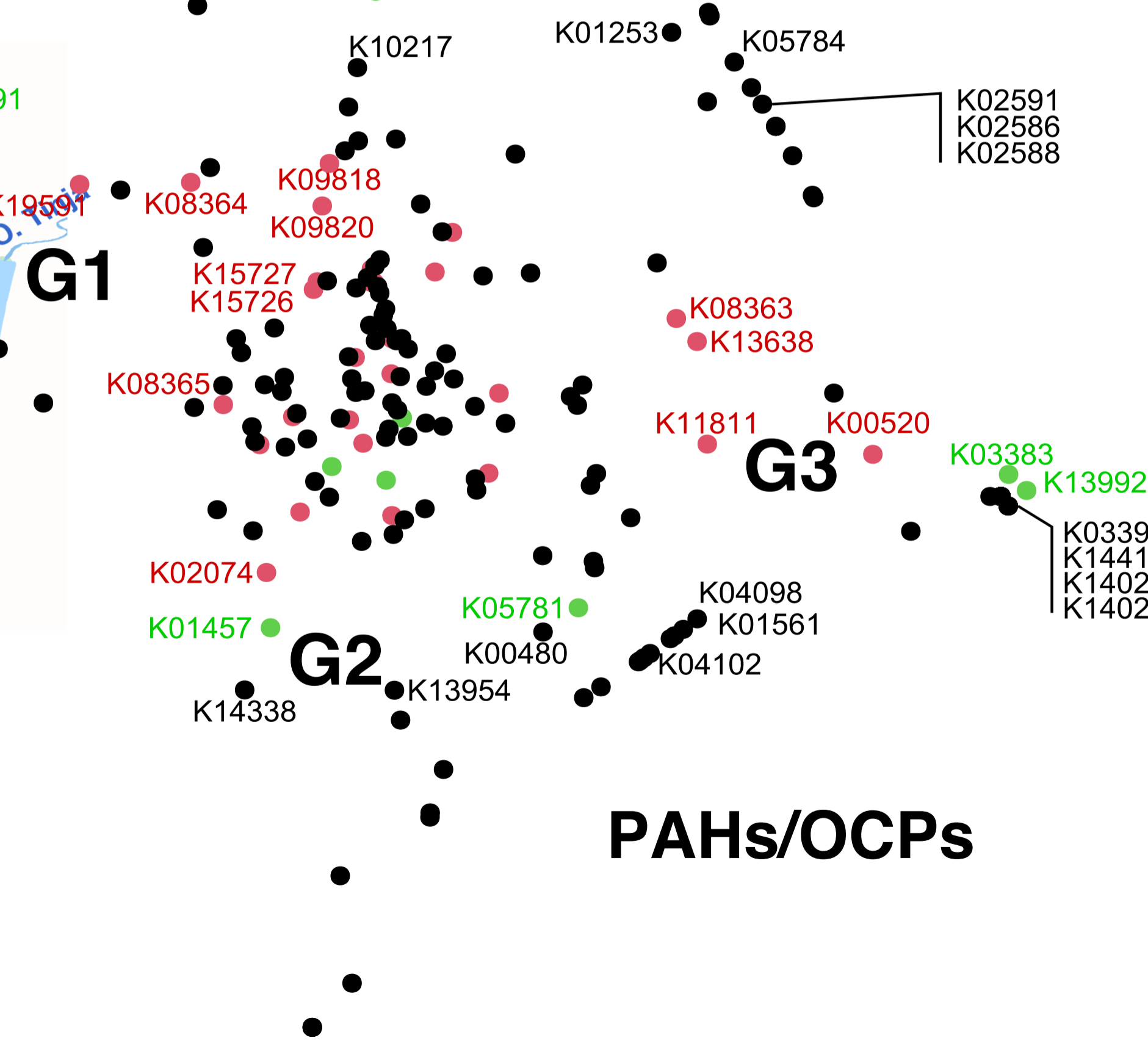




### Microbial markers



### Metals



### PAHs/OCPs