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1 **New insights in bacterial and eukaryotic diversity of microbial mats**
2 **inhabiting exploited and abandoned salterns at the Ré Island (France)**

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18

19 **ABSTRACT**

20 In order to understand the effect of human practices on microbial mats organisation, the study aimed to
21 investigate the biodiversity within microbial mats from exploited and abandoned salterns. Despite
22 several attempts, archaeal 16S rRNA gene fragment sequences were not obtained, indicating that
23 microbial mats were probably dominated by Bacteria with very low abundance of Archaea (< 1%).
24 Thus, the study compared the bacterial and meiofaunal diversity of microbial mats from abandoned
25 and exploited salterns. The higher salinity (101 ± 3.7 psu vs. 51.1 ± 0.7 psu; Welch t-test $p < 0.05$) of
26 the exploited site maintained lower bacterial diversity in comparison to the abandoned site where the
27 salinity gradient was no longer maintained. However, the microbial mats exhibited similar bacterial
28 class composition while the eukaryotic diversity was significantly higher in the exploited saltern. The
29 abandoned saltern was dominated by sulfate-reducing bacteria and Nematoda, while the exploited
30 saltern was characterized by the presence of halophilic bacteria belonging to *Marinobacter*,
31 *Salinivibrio* and *Rhodohalobacter* genera, and the larger abundance of Hypotrichia (ciliates). Such
32 bacterial and eukaryotic diversity difference might be explained by human actions for salt recovery in
33 exploited salterns such as scraping the surface of microbial mat and increasing salinity renewing the
34 microbial mat each year. Such action decreases the bacterial diversity changing the food web structure
35 that favour the presence of a larger diversity of eukaryotic organisms. Our study provides new insights
36 on microbial mat communities inhabiting salterns, especially the consequences of abandoning saltern
37 exploitation.

38

39 **Keywords:** Hypersaline environments, marine solar salterns, microbial diversity, meiofaunal diversity,
40 human exploitation and perturbation

41

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45

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47 **Availability of data and material:** Sequencing data has been deposited in sequence database.

48 **Code availability:** Not applicable

49 **1. Introduction**

50 Microbial mats develop at the sediment/water interface in various habitats (Prieto-Barajas *et al.* 2018),
51 including coastal areas (White *et al.* 2018, 2021), sand beaches (Bolhuis and Stal 2011), estuaries (Mir
52 *et al.* 1991), lakes (Jørgensen and Cohen 1977), hot springs (Dobretsov *et al.* 2011), Andin glaciers
53 (Fleming and Prufert - Bebout, 2010; Schmidt *et al.* 2017), polar region (Valdespino-Castillo *et al.*
54 2018), freshwater Lagunas (Falcón *et al.* 2020; Yanez-Montalvo *et al.* 2020), and salterns (Giani *et al.*
55 1989; Fourçans *et al.* 2004; Kolesnikov *et al.* 2017). They are complex systems of multi-layered
56 microbial communities vertically stratified where microorganisms are organized according to
57 physical-chemical gradients of light, oxygen and sulfur (Caumette *et al.* 1994), playing an important
58 role in the major biogeochemical cycles (Jørgensen and Cohen 1977; Wieland *et al.* 2003; Sánchez-
59 Baracaldo *et al.* 2021). In microbial mats, different functional microbial groups with various metabolic
60 capacities coexist at a microscale (van Gemerden 1993). They experience daily fluctuations of
61 environmental parameters resulting in variations of their vertical distribution (Fourçans *et al.* 2006,
62 2008; Martinez *et al.* 2019). Therefore, microbial mats constitute an ideal model to study microbial
63 interactions and their response to environmental changes.

64 Microbial mats developing in marine solar salterns are among the most studied (Oren 2009) for
65 examining microbial composition and their dynamics (Plominsky *et al.* 2018). Solar salterns, generally
66 found in coastal regions, are man-made habitats for salt production through solar evaporation of
67 seawater. Seawater evaporates through a series of shallow ponds, gradually increasing salinity
68 constituting extreme hypersaline environments. The resulting brine, saturated with sodium chloride,
69 reaches crystallizer ponds where salt deposits and is then collected by scraping the surface of the
70 sediment. The salt production depends on physical-chemical processes and activities of the microbial
71 mats developing in such extreme habitats, which could result in the production of several valuable
72 chemicals by microorganisms with biotechnological potentials (Gómez-Villegas, Vigara and León
73 2018). Historically, several solar salterns have been exploited along the French Atlantic coast.
74 Nowadays, many of these solar salterns have been abandoned because of loss of economic
75 profitability. At the Ré Island (France), exploited and abandoned solar salterns coexist allowing the

76 comparison of microbial mats developing under different saline fluctuating conditions but with similar
77 environmental parameters. The salt production is artisanal, handwork following traditional practices
78 with salt harvest by hands in summer. In winter the salterns are flooded with seawater, the exploitation
79 starting in spring by increasing progressively the salinity, especially in crystallizer ponds where large
80 variations in salinity are observed until reaching concentrations where salt precipitates. In contrast, the
81 salinity variation is limited in the abandoned salterns because the water entry is no regulated. It has
82 been demonstrated that the salinity fluctuations during salt production disturb the natural habitats
83 (Tran *et al.* 2019), influencing the development and the structure of the microbial mats that also
84 depend on the seasonal variations (Boujelben *et al.* 2012; Cardoso *et al.* 2019). Because only few
85 studies have investigated abandoned salterns (Cvitković *et al.* 2011; Lee *et al.* 2020), the information
86 on how the ecosystem is affected by the modifications of human practices is still scarce. The
87 comparison of the diversity within microbial mats subjected to large saline variations (exploited
88 saltern) with that of microbial mats inhabiting ponds where the saline variations are limited
89 (abandoned saltern) within the same hydrological system will allow to describe the modifications of
90 microbial communities in response to a drastic change on the human practices, which are affecting the
91 salinity fluctuations. Understanding how the human practices affect the microbial community of
92 microbial mats in solar salterns is of paramount importance providing useful information for the
93 management of microbial communities in the context of global changes.

94 In this study, we investigated the diversity within microbial mats from exploited and abandoned
95 hypersaline ponds in salterns located at the Ré Island (France). We adopted a holistic view,
96 investigating the microbial communities by 16S and 18S rRNA genes barcoding, completed by
97 microscopic observations for the eukaryotic community, in order to describe the biodiversity in
98 microbial mats and how it is affected by human-made drastic changes. Indeed, although bacterial
99 communities inhabiting microbial mats have been widely studied (Fourçans *et al.* 2006, 2008; Bolhuis
100 and Stal, 2011; Boujelben *et al.* 2012; Bolhuis *et al.*, 2014; Stal *et al.* 2019), only few studies have
101 reported on their eukaryotic community composition (Cvitković *et al.* 2011; Edgcomb *et al.* 2014),
102 resulting in a lack of knowledge in the whole diversity of microbial mats. Our results bring new

103 insights on microbial mats biodiversity modifications in response to a perturbation due to human
104 activity.

105 **2. Material and methods**

106

107 **2.1 Sampling site**

108 The microbial mats were collected in crystallizer ponds of salterns located in two different sites, but within
109 the same hydrological system, close to Ars-en-Ré (Ré Island, France; Fig. 1, the map was drawn with the R
110 library ggmap (Kahle and Wickham 2013)), allowing the investigation of abandoning saltern exploitation
111 considering similar physical-chemical parameters. The Abandoned site (46°12'44.928"N 1°30'20.123"W)
112 located in an abandoned saltern for 15 years, and the Exploited site (46°12'41.5"N 1°30'36.9"W) located in
113 an exploited saltern. The microbial mats were sampled in spring 2017. In each site, microbial mats were
114 sampled in independent biological triplicates (R1, R2 and R3) corresponding to three distinct ponds (Fig. 1).
115 Microbial mats were sampled using a PVC tube (15 cm diameter) as corer. From each core, the first 0.5 cm
116 was pushed out, from the bottom to the up, with a piston in order to collect the microbial mat of the expected
117 thickness. After homogenisation by mixing, subsamples for DNA microbial analysis were dispatched in 2 mL
118 cryotubes flash frozen in liquid nitrogen and then stored at -80°C. Meiofauna abundance and group
119 composition were obtained from another core. Sediment (60 mL) was preserved in absolute ethanol
120 (vol/vol).

121 The water salinity (Practical Salinity Unit, psu corresponding to g/L) and the water temperature (°C) were
122 measured in triplicate in each sampling spot with a multiparameter probe (pHenomenal® MU 6100H,
123 VWRTM, USA).

124

125 **2.2 DNA extraction and Illumina sequencing**

126 Microbial mat samples were ground in liquid nitrogen with a mortar and a pestle as previously described
127 (Fourçans et al., 2008). Then, DNA was extracted from 0.25 g subsamples using the DNeasy® PowerSoil kit
128 (Qiagen) according to the manufacturer's instructions, with a slight modification at the homogenization step,
129 by using a Precellys homogenizer (Bertin Instruments).

130 The V3-V4 region of the bacterial 16S rRNA genes (460 bp) was amplified using the primers PCR1F_460
131 (5'-ACGGRAGGCAGCAG-3') and PCR1R_460 (5'-TACCAGGGTATCTAATCCT-3') (Klindworth *et al.*
132 2013). The Primers 519F (5'-GCCGCCGCGGTAA-3') and 915R (5'-GTGCTCCCCCGCCAATTC-3') were
133 used to amplify the V3-V5 region (400 pb) of the archaeal 16S rRNA gene (Hugoni *et al.* 2015). Polymerase

134 chain reaction (PCR) mix consisted in 5 μ L of MTP Taq DNA polymerase (5 U/ μ L) with 1 μ L MTP Taq
135 Buffer (10X) (Sigma-Aldrich), 1 μ L of dNTP (10 mM), 1.25 μ L of each primer (20 μ M) and 10 ng of
136 genomic DNA, in a final volume of 50 μ L (adjusted with distilled water). All amplifications were performed
137 on a Labcycler (SensoQuest) using the following PCR program: 2 min at 94°C, 30 cycles of 60 s at 94°C, 40
138 s at 65°C for Bacteria (40s at 57°C for Archaea), and 30 s at 72°C, and finally, 10 min at 72°C.

139 The V4 region (390 bp) of the 18S rRNA genes was amplified using the universal primer 515F (5'-
140 GTGYCAGCMGCCGCGGTA-3') (Caporaso *et al.* 2011) and the eukaryotic primer 951R (5'-
141 TTGGYRAATGCTTTTCGC-3') (Lepère *et al.* 2016). PCR mix consisted in 27.5 μ L of AmpliTaq Gold[®] 360
142 master mix (Applied Biosystems), 1.1 μ L of each primer (20 μ M) and 5.5 μ L of genomic DNA, in a final
143 volume of 55 μ L (adjusted with distilled water). All amplifications were performed on a Veriti 96 Well
144 Thermal Cycler (Applied Biosystem) using the following PCR program: 10 min at 95°C, 35 cycles of 30 s at
145 95°C, 30 s at 55°C and 45 s at 72°C, and finally, 10 min at 72°C.

146 All PCR were performed in triplicate for each sample and sequenced separately in order to obtain technical
147 replicates in order to consider the PCR bias. Illumina sequencing was performed by the NED team
148 (UMR1388 GenPhySE) and the GeT core facility (Toulouse, France), using Illumina MiSeq technology
149 (paired-end 2 x 250 bp). The complete dataset was deposited in the NCBI Sequence Read Archive (SRA)
150 database under SRA accession number PRJNA627371 for eukaryotic dataset and PRJNA627173 for
151 bacterial dataset.

152

153 **2.3 Sequence processing**

154 Bioinformatic processing for DNA sequences were performed using QIIME 2 2019.4 (Bolyen *et al.* 2019).
155 The same process was followed for 16S (Bacteria) and 18S (Eukaryote) rRNA gene sequences. Raw
156 sequences were demultiplexed and then filtered, denoised, merged and grouped in non-chimeric sequences
157 with DADA 2 (Callahan *et al.* 2016) followed by a singleton filtering. The taxonomic affiliation was
158 performed against the Silva database v132 (Quast *et al.* 2012; Yilmaz *et al.* 2013) with 97% of similarities in
159 order to compare the biodiversity obtained in previous studies, as it is used in most studies exploring
160 biodiversity in microbial mats (Cardoso *et al.* 2019; Sierra *et al.* 2020; Vogt *et al.* 2018). Additionally, Silva
161 database allows to analyse the three domains of life (Bacteria, Archaea, and Eukarya) while the other
162 databases (e.g. Greengenes, RDP) will not (Balvočiūtė and Huson 2017). The non-affiliated sequences were

163 excluded. The rarefaction was done at 5,530 and 22,274 sequences per sample for bacteria and eukaryote
164 respectively (Supplementary materials, Fig. S1.), which correspond to the lowest number of sequences
165 considering the PCR replicates (data not shown).

166

167 **2.4 Meiofauna characterization**

168 Microbial mat was sieved through 50 µm before staining with rose Bengal and observation under a binocular
169 loupe (magnification x30, Zeiss). A sample splitter (Motoda box as Rzeznik-Orignac *et al.*, 2003) was used
170 to obtain an aliquot containing at least 100 individual nematodes for abundance estimation. The abundance
171 of other meiobenthic taxa (i.e. copepods and ostracodes) was too low to be evaluated in split samples and,
172 therefore, was quantified using whole samples. Abundances were expressed as individuals per cubic
173 centimetre (ind. cm⁻³).

174

175 **2.5 Statistical analysis**

176 Statistical analyses were performed using Rstudio software (R version 3.6.3, (2020-02-29), R Core Team,
177 2020). The water temperature between the two sites was compared with a Student t-test because the
178 conditions of application (normality of data and variance independence) were verified and with a Welch two
179 sample t-test for salinity because the independence of variances was not verified. Then, the difference in the
180 relative microbial abundance was investigated between the abandoned and exploited sites. The biom file
181 produced by the bioinformatic analysis and the table of environmental data were merged into a single R
182 package using “phyloseq” (McMurdie and Holmes 2013). The statistical analyses were performed on the
183 average of the PCR triplicates (technical replicates) for each sample in order to consider the PCR bias. The
184 alpha diversity was calculated by two indices, the richness (based on presence/absence of Amplicon
185 Sequence Variants (ASVs)) and the Shannon index (according to the relative abundance of ASVs). The
186 abundances were compared at different taxonomic levels with a Student t-test if the conditions of application
187 (normality of data and variance independence) were verified, or with a Welch two sample t-test if the
188 normality was verified but not the independence of variances and with a Wilcoxon-Mann-Whitney test if any
189 condition was respected. The beta diversity between sites was then analysed with a principal coordinate
190 analysis (PCoA) of Bray-Curtis distance matrices. A dendrogram of each replicate of the sites was also done
191 with Bray-Curtis distance matrices. The contribution of each ASV explaining the differences between sites

192 was defined by SIMPER (SIMilarity PERcentages) based on Bray-Curtis distance measure. Linear
193 discriminant analysis effect size (LEfSe) (Segata *et al.* 2011) on the 50 more abundant ASVs was done on
194 Galaxy web application to determine bacterial genera or eukaryotic families biomarkers of each site. The
195 non-parametric Kruskal-Wallis sum-rank test ($\alpha = 0.05$) was performed to detect taxa with significant
196 differential abundance. The biological consistency was investigated by performing a pairwise Wilcoxon test
197 ($\alpha = 0.05$). A linear discriminant analysis (LDA) threshold score of 2.0 was applied.

198

199

200 **3. Results and discussion**

201

202 **3.1 Description of the environmental parameters and the microbial mats**

203 The water temperature and salinity were significantly higher in exploited site ($25 \pm 1^\circ\text{C}$; 101 ± 3.7 psu) than
204 in the abandoned site ($23 \pm 0.4^\circ\text{C}$; 51.1 ± 0.7 psu) (Student t-test for temperature, $p < 0.05$; Welch two sample
205 t-test for salinity, $p < 0.05$), while in both sites the water was at pH 8. Such results were expected as salinity
206 increases in ponds for salt production without being subjected to desiccation, while seawater entering into
207 the abandoned site maintains a lower salinity. The microbial mats showed different morphological aspects,
208 particularly in their thickness that may be explained by the scraping during salt recovery, resulting in a
209 thinner microbial mat in the exploited site. Also, it has been shown that salinity affect exopolysaccharide
210 (EPS) production, that in turn might affect the mat structure and properties (Decho and Gutierrez 2017).
211 Such observation suggested that the environmental conditions may affect the microbial mats as observed in
212 diverse coastal ecosystems including sediments (Pringault *et al.* 2008; Duran *et al.* 2015), estuarine mudflats
213 (Chronopoulou *et al.* 2013) and hypersaline microbial mats (Bordenave *et al.* 2004).

214

215 **3.2 Microbial and meiofaunal community diversity in abandoned and exploited salterns**

216 Despite several attempts, we were unable to obtain archaeal 16S rRNA gene fragment sequences. Although
217 DNA extraction and PCR amplification bias cannot be excluded, this result was in accordance with previous
218 studies showing that benthic mats in hypersaline ponds are dominated by bacteria and are even almost
219 exclusively bacterial (Bolhuis and Stal, 2011; Bolhuis *et al.*, 2014; Stal *et al.*, 2019), with very low
220 abundance of Archaea below 1%, especially within the upper part (0.5 cm) of the mat (Bolhuis and Stal,

221 2011) as analysed in this study. The flooding each winter, after salt recovery, induces the fluctuation of
222 environmental parameters resulting on the renewing of the microbial mats that may also explain the fact that
223 Archaea were not detected. Indeed, Archaea have been demonstrated to be sensitive to drastic modifications
224 of environmental parameters (Zhao *et al.*, 2020) probably preventing their development within microbial
225 mats. Because Archaea have been shown to play a critical role in ecosystem function (Wong *et al.* 2017),
226 further efforts with optimised molecular methods and tools will be beneficial to describe the archaeal
227 diversity in microbial mats. Particularly, it is worth to note that our attempt to target Archaea was based on
228 the utilisation of only a set of primers, several sets of primers have been developed allowing to extend the
229 capacity to reveal Archaea diversity (Bahram *et al.* 2019) that will help to detect Archaea in the microbial
230 mats. Thus, our study focuses on the bacterial and eukaryotic components of the microbial mats inhabiting
231 the abandoned and exploited saltern sites.

232 The composition of bacterial and eukaryotic communities was determined by high throughput sequencing in
233 order to compare microbial mats of the abandoned salterns to that of exploited salterns. The number of raw
234 sequences ranged between $30,243 \pm 4,898$ (abandoned site) and $19,200 \pm 2,626$ (exploited site) for Bacteria,
235 and between $50,119 \pm 720$ (abandoned site) and $39,684 \pm 1,408$ (exploited site) for Eukaryota
236 (Supplementary materials, Table S1). After filtering, the rarefaction provided a random subsample of 5,530
237 bacterial sequences and 22,274 eukaryotic sequences per sample (Supplementary materials, Table S1).

238 The bacterial richness observed for the microbial mats inhabiting the abandoned site was in the same range
239 (between 200-700 ASVs) to that observed for other coastal ecosystems (Dillon *et al.* 2013; Cardoso *et al.*
240 2019). The bacterial Shannon indexes of the abandoned and the exploited sites (5.75 and 4.6, respectively)
241 were higher than that reported for prokaryotic communities from solar salterns (Dillon *et al.* 2013) and
242 spring coastal microbial mats (Cardoso *et al.* 2019) (Fig. 2).

243 For Bacteria, the richness and the Shannon index were significantly higher (Student t-test, $p < 0.05$) in the
244 abandoned site (643 ± 60 ASVs and 5.73 ± 0.09) than in the exploited site (265 ± 75 ASVs and 4.77 ± 0.27 ;
245 Fig. 2A, 2B). Interestingly, for Eukaryota, the opposite was observed, the richness and the Shannon index
246 being significantly higher (Student t-test, $p < 0.05$) in the exploited site (187 ± 26 ASVs and 3.03 ± 0.29) than
247 in the abandoned site (121 ± 25 ASVs and 1.53 ± 0.33 ; Fig. 2C, 2D). Such observation was consistent with

248 previous reports indicating that salinity is a main driver for bacterial community diversity (Fei Xi *et al.* 2014;
249 Jeanbille *et al.* 2016a; Ben Salem *et al.* 2019) and for the meiofaunal abundance and species composition
250 (Coull 1999). It has been demonstrated that the richness and abundance of bacteria and eukaryotes decrease
251 along a gradient of salinity because the conditions become extreme with increasing salinity (Nubel *et al.*
252 2000; Pedrós-Alió *et al.* 2000; Benlloch *et al.* 2002; Estrada *et al.* 2004). For eukaryotes, some populations
253 have been reported dominant in high salt concentration (above seawater salinity, 35 psu) ecosystems
254 (Elloumi *et al.* 2009). Interestingly, the eukaryotic diversity was higher in the more salted site (exploited) in
255 our study.

256 The human actions for salt exploitation, which include regular scraping for salt recovery and microbial mat
257 removal together with the fluctuations of environmental parameters (Wieland *et al.* 2003), are probably
258 responsible for the decreased bacterial diversity observed in the exploited site in comparison to the
259 abandoned site. Indeed, the abandoned site is not disturbed by human actions, which results to an ecosystem
260 with weak fluctuations that is considered as an ecosystem with less selective pressure for bacterial
261 communities (Graham *et al.* 2016). In contrast, the increasing of eukaryotic diversity with salinity has never
262 been reported before for hypersaline environments (Pillay and Perissinotto 2009; Heidelberg *et al.* 2013).
263 Other factors have been reported to influence the composition of the eukaryotic population like
264 hydrodynamic (Kapusta *et al.* 2005), sediment stability (Kapusta *et al.* 2005), sediment particle size (Coull
265 1999), temperature (Coull 1999; Kapusta *et al.* 2005), depth (Baguley *et al.* 2006), physical disturbance
266 (Austen and Widdicombe 2006), nutrient enrichment (Austen and Widdicombe 2006) and long-term
267 anthropogenic impacts (Boldina, Beninger and Coz 2014). Nevertheless, we assume that physical
268 disturbance and long-term anthropogenic actions were the most probable factors driving eukaryotic
269 communities in our study since salt workers in Ré Island follow a traditional exploitation removing the
270 accumulated mud each winter as described for the Aveiro salterns (Rodrigues *et al.* 2011). More information
271 about the physical-chemical parameters such as pH, dissolved oxygen concentration and water renewable
272 rates will be necessary to determine the factor influencing the eukaryotic composition.

273

274 **3.3 Bacterial and meiofaunal community composition of microbial mats inhabiting abandoned and**
275 **exploited salterns**

276 The first 0.5 cm of microbial mats from both sites were dominated by the same seven bacterial phyla with
277 high abundance of Proteobacteria and Bacteroidetes (Fig. 3). This phyla composition was comparable to that
278 of coastal microbial mats where Cyanobacteria, Proteobacteria and Bacteroidetes are dominant (Cardoso *et al.*
279 *et al.* 2019) as well as in most hypersaline microbial mats (Pal *et al.* 2020). Bacteroidetes and Proteobacteria,
280 described as dominant phyla in the water and sediment of other salterns (Boujelben *et al.* 2012; Lee *et al.*
281 2020), are known to play important roles in carbon and nitrogen cycles (Bernhard, Marshall and Yiannos
282 2012; Wong *et al.* 2015). Only Gammaproteobacteria showed significant differences between the studied
283 sites: they were significantly more abundant in the exploited site than in the abandoned site (Student t-test,
284 $p < 0.05$). Such Gammaproteobacteria abundance discrepancy between both sites might be explained by the
285 fact that Gammaproteobacteria class has been reported to be composed of pioneering microorganisms during
286 the biofilm formation (Lee *et al.* 2008), which is in accordance with the fact that the microbial mat is
287 renewed every year in the exploited site. Generally, Cyanobacteria are found dominant in microbial mat
288 composition (Fourçans *et al.* 2004, 2006, 2008; Bolhuis and Stal, 2011; Boujelben *et al.* 2012; Bolhuis *et al.*,
289 2014; Stal *et al.* 2019). Surprisingly, they accounted for less than 1% of the total phyla abundance in our
290 study although that underestimation of Cyanobacteria cannot be excluded due to technical bias such as DNA
291 extraction and primers efficiencies as previously reported (Fourçans *et al.* 2004).

292 Assuming that the physical-chemical parameters of the abandoned saltern, particularly the salinity, tend to be
293 similar to those found at the near oceanic coast, we expect that the microbial mat composition will be close
294 to that observed in microbial mats inhabiting such coastal areas. The microbial composition of the coastal
295 sediment from Ré Island has not yet been described. However, Proteobacteria and Planctomycetes were
296 found to be dominant in the first centimetre of sediments from Marennes-Oléron Bay, located about 50 km
297 from Ré Island, while Bacteroidetes was the fourth dominant phylum (Lavergne 2014), which differs from
298 the bacterial phyla composition of microbial mats from both abandoned and exploited sites. Such observation
299 suggested that the abandonment period (15 years) was not long enough for erasing the effect of saltern
300 exploitation to reach the bacterial composition observed in coastal sediments, probably because the salinity
301 in both abandoned and exploited sites exceeds regularly the seawater salinity (> 35 psu). To the best of our
302 knowledge, only two studies have reported the reorganization of microbial communities after the
303 abandonment of salterns exploitation (Bernhard, Marshall and Yiannos 2012; Lee *et al.* 2020). These studies
304 concluded that the physical-chemical parameters were similar to that observed at the coast only one year

305 after the natural collapse of embankments, but the bacterial composition remained different for more than 30
306 years (Bernhard, Marshall and Yiannos 2012; Lee *et al.* 2020). It is important to mention that in our study the
307 salterns were still intact maintaining the salinity gradient, which drives bacterial community structure.
308 Further long time-series analyses comparing microbial communities in different locations at Ré Island are
309 still required to determine whether the microbial community reach the structure observed in the coast.

310 The eukaryotic community in the abandoned site was dominated by only three phyla (abundance above 1%)
311 while the exploited site was characterized by a large eukaryotic diversity with six main phyla identified (Fig.
312 4). Metazoa dominated the eukaryotic community in the abandoned site, being significantly more abundant
313 (Student t-test, $p < 0.05$) than in the exploited site (Fig. 4). The presence of Metazoa in both sites was not
314 surprising since they are often found in marine sediments, which are usually dominated by Nematoda
315 followed by Platyhelminthes and Arthropoda (Fonseca *et al.* 2014; López-Escardó *et al.* 2018). All the phyla
316 identified have been ever seen in other exploited salterns (Feazel *et al.* 2008; Stock *et al.* 2012) and in
317 abandoned salterns (Cvitković *et al.* 2011) but, to our knowledge, no studies compared the eukaryotic
318 composition in these two types of salterns.

319 The more important eukaryotic diversity observed in the exploited site, in comparison to that of the
320 abandoned site, might be explained by the human action of scratching the surface of the microbial mat,
321 which probably prevents colonization by a single eukaryotic population.

322 Because it is known that molecular analysis has inherent bias derived from DNA extraction efficiency and
323 primers specificity (Tedersoo, Tooming-Klunderud and Anslan 2018), the eukaryotic diversity was
324 completed by meiofaunal microscopic observations. The presence of Acari, Polychaeta, insect larvae and
325 *Copepoda nauplii* was observed that were not detected by molecular analysis (Supplementary materials, Fig.
326 S2). Consistently with the molecular analysis, Nematoda largely dominated (>99%) the observable
327 meiofaunal community of the abandoned site (Supplementary materials, Fig. S2). But, in contrast to the
328 molecular analysis, Nematoda were also observed (around 69%) together with adult Copepods (26%), Acari
329 (4%), insect larvae (2%) and Polychaeta (2%) in the exploited site (Supplementary materials, Fig. S2). Thus,
330 we advocate that the combination of molecular techniques with microscopic observations will help to obtain
331 a more comprehensive and accurate analysis of eukaryotes species diversity in the environmental samples.

332

333 **3.4 Comparison of bacterial and meiofaunal communities from abandoned site and from exploited site**

334 The comparison of the bacterial and eukaryotic communities between the abandoned site and the exploited
335 site by principal coordinate analysis (PCoA) showed a clear separation between the sites along the PCo1 axis
336 explaining around 47% of the data distribution for the bacterial community (Fig. 5A) and 92% for the
337 eukaryotic community (Fig. 5B). Noteworthy, the PCoA analysis showed a heterogeneity within the
338 exploited site with a dispersion of the triplicates along the PCo2 axis explaining around 21% of the data
339 distribution for bacteria (Fig. 5A) and around 5% for eukaryotes (Fig. 5B). However, cluster analysis based
340 on Bray-Curtis distances confirmed that the bacterial and eukaryotic communities of the triplicates from the
341 abandoned site were clustered together, clearly separated from the cluster formed by the bacterial and
342 eukaryotic communities of the exploited site (Fig. 5C and 5D). The difference of the bacterial and eukaryotic
343 communities between both sites was further confirmed by SIMPER analysis showing overall dissimilarity
344 for bacterial community compositions (SIMPER: 50%) as well as for eukaryotic community compositions
345 (SIMPER: 72%).

346 Accordingly, LEfSe analyses revealed taxa, bacterial and eukaryotic, differentially abundant between both
347 sites (Fig. 6), which represent site-specific biomarkers as previously suggested in studies identifying
348 environmental and contaminant microbial biomarkers (Segata *et al.* 2011; Jeanbille *et al.* 2016b). The
349 abandoned site was characterized by 11 bacterial and 5 eukaryotic biomarkers (Fig. 6). Interestingly, ASVs
350 related to *Loktanella* (Alphaproteobacteria) and Desulfobacterales, which are usually found in abandoned
351 salterns and tidal flats (Lee *et al.* 2020), were identified among the bacterial biomarkers (Fig. 6A). The
352 Desulfobacterales, sulfate reducing bacteria generally predominant in sulfate-rich sediment (Ruff *et al.*
353 2015), have been shown to influence the microbial re-colonization of abandoned salterns (Lee *et al.* 2020).
354 Similarly, the Alphaproteobacteria *Loktanella*, has been described as a pioneer bacterium in marine biofilm
355 formation (Lee *et al.* 2008). The identification of such biomarkers suggested that the bacterial community at
356 the abandoned site was undergoing a re-organization. Regarding the eukaryotic biomarkers, ASVs related to
357 Arthropoda, Nematoda and Platyhelminthes belonging both to Metazoa as specific members of abandoned
358 site (Fig. 6B). It is known that Nematoda dominate in marine sediments, followed by Platyhelminthes,
359 Arthropoda and a random assemblage of Gastrotricha, Annelida, Mollusca... (Fonseca *et al.* 2014; López-
360 Escardó *et al.* 2018). This observation was in accordance with our hypothesis that the microbial composition
361 in the abandoned site would tend to be similar to that found on the coast.

362 According to the LEfSe analyses, the exploited site was characterized by 9 bacterial and 14 eukaryotic
363 biomarkers (Fig. 6). Consistent with the high salinity prevailing at the exploited site, ASVs related to
364 halophilic bacterial genera were identified as biomarkers (Fig. 6A) such as *Marinobacter*, *Salinivibrio* and
365 *Rhodohalobacter* that are usually found in hypersaline environments (Gorshkova 2003; Duran 2010; Kim *et*
366 *al.* 2017; Xia *et al.* 2017; López-Hermoso *et al.* 2018; de la Haba *et al.* 2019). Such information showed the
367 salinity impact of the microbial mat organisation, further highlighting the differences the contrasted
368 organization with the microbial mat from the abandoned salterns.

369 The specific eukaryotic ASVs identified in the exploited site were more diverse than that identified in the
370 abandoned site. For example, ASVs related to the Ciliophora phylum were detected in both sites but the
371 populations were different at the class and subclass levels (Fig. 6B). Choreotrichia, Haptoria and Hypotrichia
372 sub-classes, and Phyllopharyngea class were found to be significantly more abundant in the exploited site,
373 while ASVs related to Litostomatea class were significantly predominant in the abandoned site (Fig. 6B).
374 Such observation was in accordance with previous report showing that members of the Ciliophora phylum
375 (ciliates) were influenced by the salinity, their abundance and biomass increasing with salinity together with
376 a modification in their composition (Nche-Fambo, Tirok and Scharler 2016). Additionally, members of the
377 Choreotrichia subclass have been reported dominant in saline environments with salinities reaching up to 64
378 psu (Nche-Fambo, Tirok and Scharler 2016). The other ASVs found significantly dominant in the exploited
379 site were related to eukaryotic phototrophs related to the Chlorophyta phylum (*Dunaliella*), Dinophyceae
380 class (Dinoflagellates), Haptista phylum (*Isochrysis* and *Pleurochrysis*), and Diatomea (Fig. 6B), indicating
381 that the exploited site was dominated by eukaryotic phototrophs in comparison to the abandoned site. In
382 contrast, the abandoned site was characterized by the presence of ASVs related to Nematoda, Arthropoda,
383 Platyhelminthes and Ciliophora (Litostomatea) phyla, and Craspedida order, which were found significantly
384 abundant (Fig. 6B). Such differences in eukaryotic composition between both sites suggested an important
385 influence of the salinity in the organization of eukaryotic community that in turn might provoke changes in
386 the food web as previously reported along a salinity gradient (Pedrós-Alió *et al.*, 2000). It is likely that the
387 low diversity of preys on the abandoned site (bacteria and ciliates) favoured the development of Metazoa,
388 explaining that Metazoa related ASVs (Nematoda, Arthropoda, and Platyhelminthes) were found
389 significantly more abundant than in the exploited site. Indeed, the Metazoa related ASVs (Nematoda,
390 Arthropoda, and Platyhelminthes) have been shown to be able to feed on bacteria (De Mesel *et al.* 2004;

391 Feazel *et al.* 2008; Hubas *et al.* 2010), diatoms (Montagna 1984), phytobenthos (algae and diatoms; Cowles
392 *et al.*, 1988), ciliates (Berk *et al.* 1977), and dinoflagellates (Cowles, Olson and Chisholm 1988; Turner
393 2004). In contrast, in the exploited site, the higher diversity of Ciliophora (ciliates) related ASVs together
394 with large diversity of eukaryotic phototrophs (Diatoms, Dinoflagellata and Chlorophyta; (Cupp 1943; Lewis
395 and McCourt 2004; Jordan 2012) indicated a larger diversity of preys than in the abandoned site. The ciliates
396 are well known as major predator for bacteria (Sherr and Sherr 1987; Parry 2004) but they can also feed
397 flagellates and other ciliates (Bernard and Rassoulzadegan 1990; Dolan and Coats 1991). It is likely that the
398 exploitation of the saltern resulted in more extreme conditions (higher salinity) reducing the bacterial
399 diversity with concomitant modification in food web structure.

400

401 **4. Conclusion**

402 After stopping the exploitation of the salterns, the abandoned site was characterized by lower salinity with
403 limited variations in comparison to the exploited site. Such conditions favoured the installation of a more
404 diverse bacterial community in the abandoned site while the eukaryotic diversity was lower with the
405 dominance of Nematoda. The presence of site specific ASVs, being significantly more abundant according to
406 the site (biomarkers), suggested a re-organisation of the microbial mat in the abandoned site with a structure
407 tending to get closer to that found in coastal areas. In contrast, the more extreme conditions at the exploited
408 site, characterized by salinity variations, resulted in reduced bacterial diversity with a modification of the
409 food web structure. The exploited site presented halophilic bacteria and a larger diversity of eukaryotic
410 metabolisms. It is likely that the human exploitation of salterns constrains the microbial composition of the
411 microbial mats, which undergo perceptible modifications several years after the abandonment of the salterns.
412 A monitoring of the microbial mats during several years (at least 15 years) on the abandoned salterns is
413 necessary to understand precisely how this structure could be resilient face to human activities.

414

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430

431 ***Conflicts of interest***

432 None declared.

433

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697 **Figure caption**

698 **Figure 1: Localisation of the sampling sites at the Ré Island (France).** Microbial mats were sampled in
699 abandoned (red point) and exploited (green point) sites in independent triplicates corresponding to three
700 ponds in each site.

701
702 **Figure 2: Diversity indexes for bacterial (A, B) and eukaryotic (C, D) communities in the abandoned**
703 **(red) and exploited (green) sites.** The boxplots show observed richness (A, C) and Shannon (B, D) indexes
704 determined at the ASV level from 16S (Bacteria) and 18S (Eukarya) rRNA gene sequences. Different letters
705 indicate significant differences based on Student t-test at $p < 0.05$ ($n = 3$).

706
707 **Figure 3: Bacterial community compositions at the phylum level in the abandoned and exploited sites.**
708 The bar plots represent each replicate, presenting the phyla with a relative abundance above 1%. The
709 class level within Proteobacteria phylum is shown.

710
711 **Figure 4: Eukaryotic community compositions at the phylum level in the abandoned and exploited**
712 **sites.** The bar plots represent each replicate, presenting the phyla with a relative abundance above 1%.

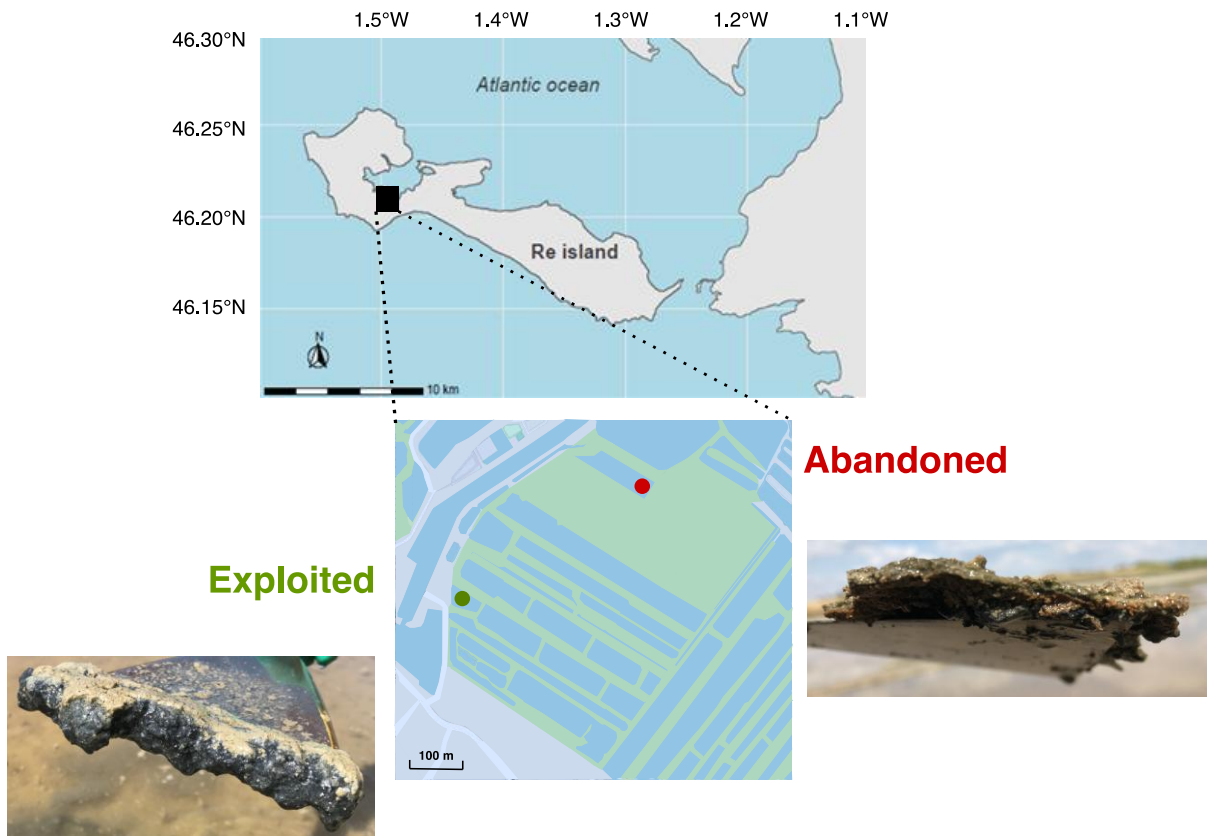
713
714 **Figure 5: Comparison of bacterial and eukaryotic communities inhabiting the abandoned (red) and**
715 **exploited (green) sites.** (A, B) Principal coordinate analysis (PCoA) comparing bacterial (A) and eukaryotic
716 (B) communities. (C, D) Clustering analysis based on Bray-Curtis distances.

717
718 **Figure 6: Comparison of bacterial (A) and eukaryotic (B) communities inhabiting the abandoned (red)**
719 **and exploited (green) sites by linear discriminant analysis effect size (LEfSe).** The analysis was
720 performed with the 50 more abundant bacterial and eukaryotic ASVs of each site.

721

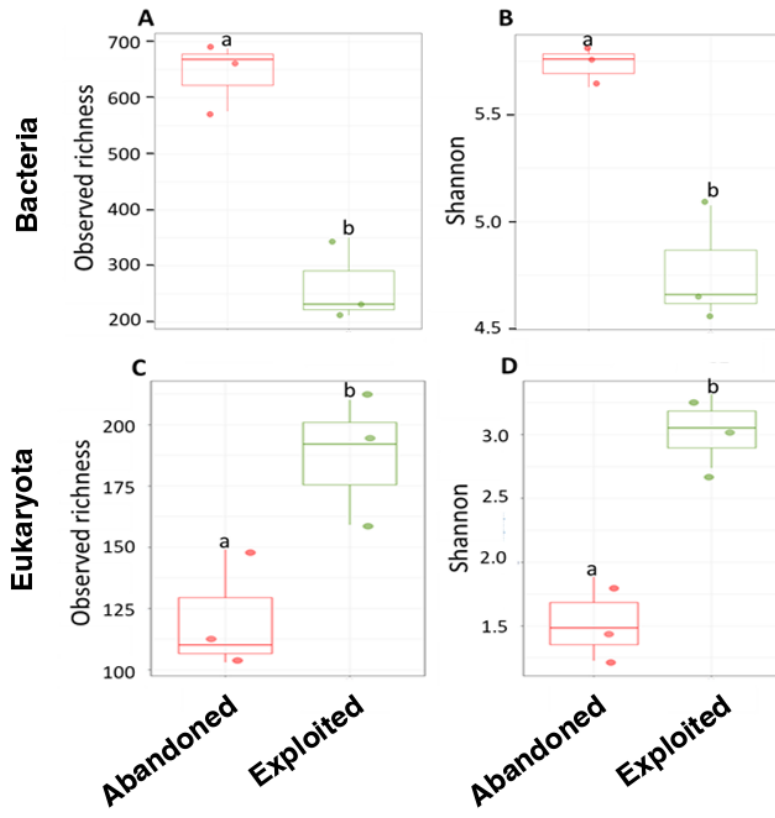
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723 Figure 1



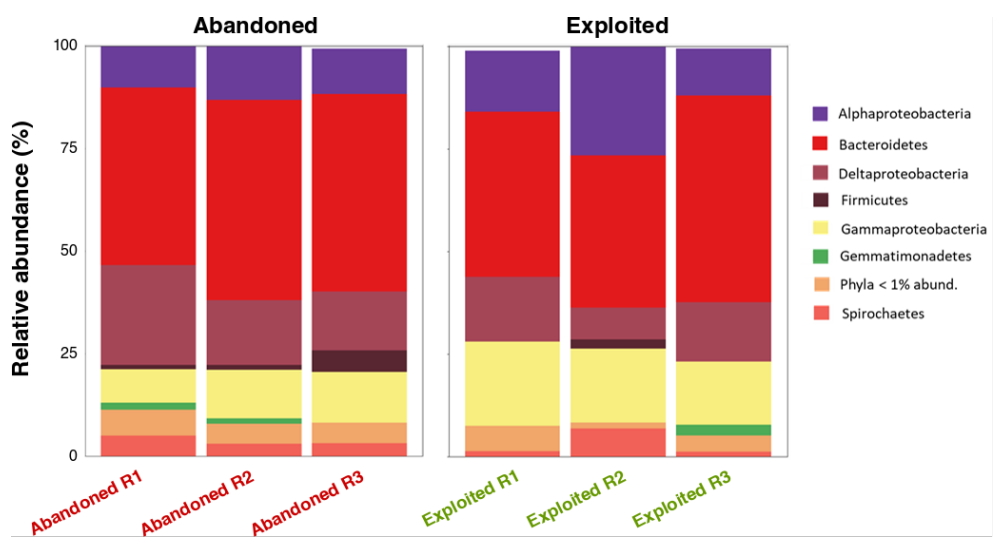
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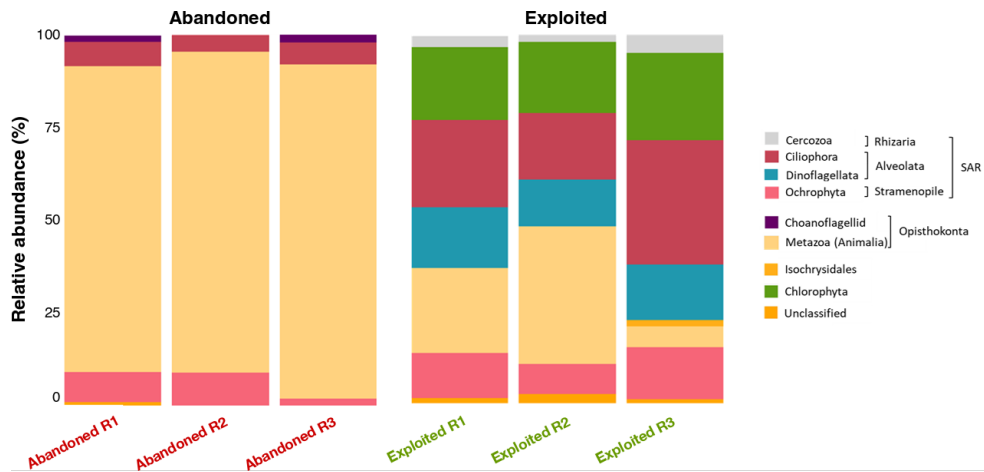


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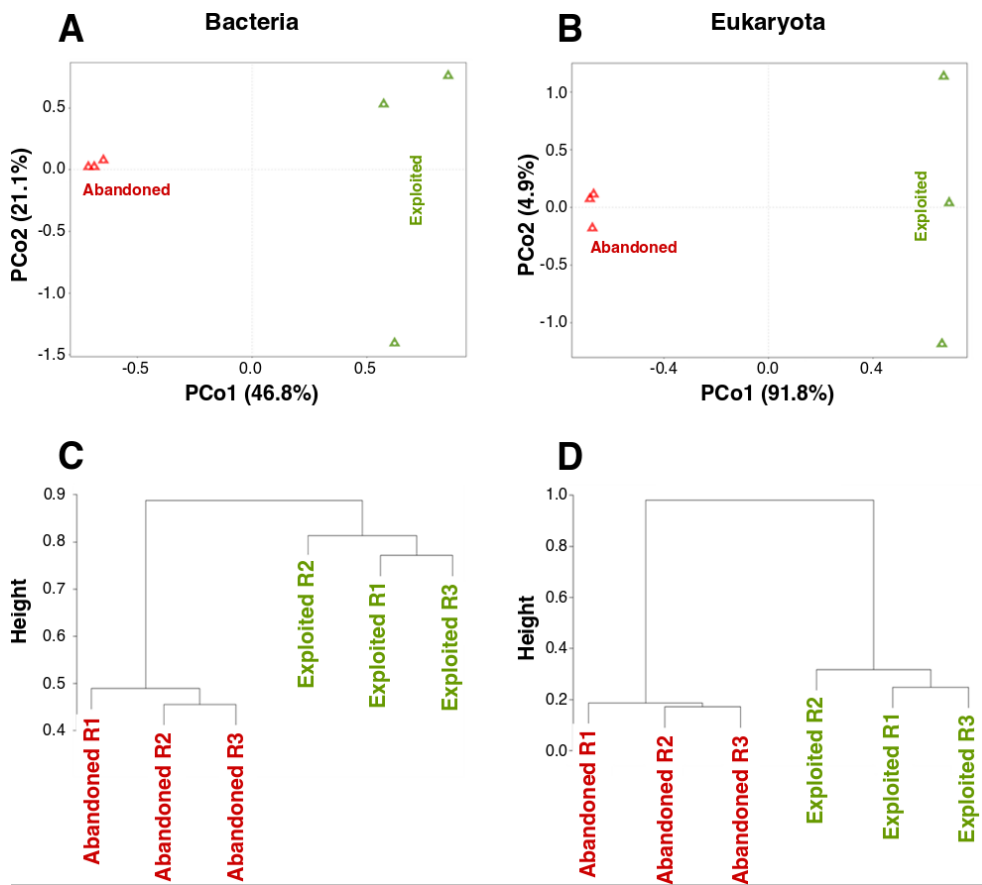
732 Figure 4

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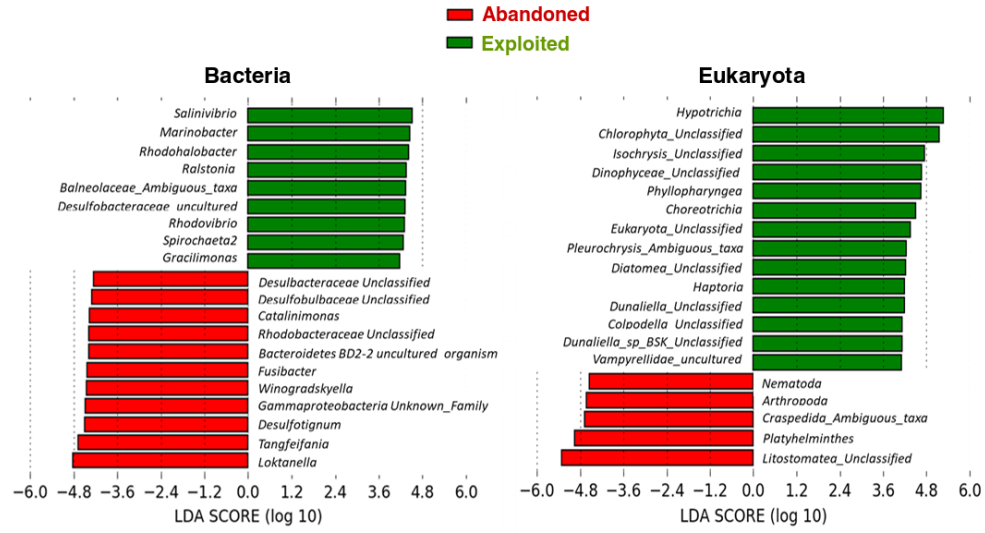
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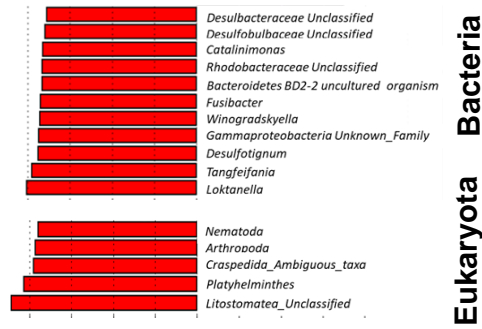
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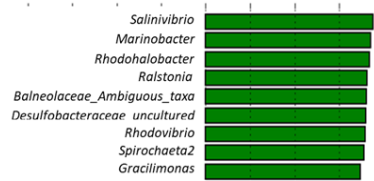
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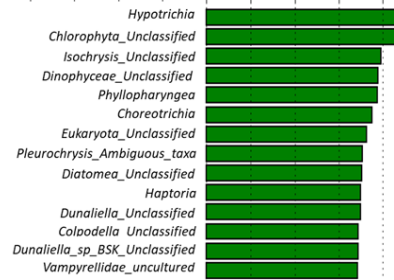
**Microbial bio-markers
for abandoned saltern**



Bacteria



Eukaryota



**Microbial bio-markers
for exploited saltern**

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