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ABSTRACT

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exploited site maintained lower bacterial diversity in comparison to the abandoned site where the salinity gradient was no longer maintained.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

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

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Code availability: Not applicable

1. Introduction

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

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

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

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

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

2. Material and methods

2.1 Sampling site

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

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

2.2 DNA extraction and Illumina sequencing

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

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

chain reaction (PCR) mix consisted in 5 µL of MTP Taq DNA polymerase (5 U/µL) with 1 µL MTP Taq Buffer (10X) (Sigma-Aldrich), 1 µL of dNTP (10 mM), 1.25 µL of each primer (20 µM) and 10 ng of genomic DNA, in a final volume of 50 µL (adjusted with distilled water). All amplifications were performed on a Labcycler (SensoQuest) using the following PCR program: 2 min at 94°C, 30 cycles of 60 s at 94°C, 40 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.

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

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

2.3 Sequence processing

Bioinformatic processing for DNA sequences were performed using QIIME 2 2019.4 (Bolyen *et al.* 2019). The same process was followed for 16S (Bacteria) and 18S (Eukaryote) rRNA gene sequences. Raw sequences were demultiplexed and then filtered, denoised, merged and grouped in non-chimeric sequences with DADA 2 (Callahan *et al.* 2016) followed by a singleton filtering. The taxonomic affiliation was performed against the Silva database v132 (Quast *et al.* 2012; Yilmaz *et al.* 2013) with 97% of similarities in order to compare the biodiversity obtained in previous studies, as it is used in most studies exploring biodiversity in microbial mats (Cardoso *et al.* 2019; Sierra *et al.* 2020; Vogt *et al.* 2018). Additionally, Silva database allows to analyse the three domains of life (Bacteria, Archaea, and Eukarya) while the other 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 dendogram 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

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

3. Results and discussion

3.1 Description of the environmental parameters and the microbial mats

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

3.2 Microbial and meiofaunal community diversity in abandoned and exploited salterns

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4. Conclusion

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

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Conflicts of interest

None declared.

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Figure caption

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

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

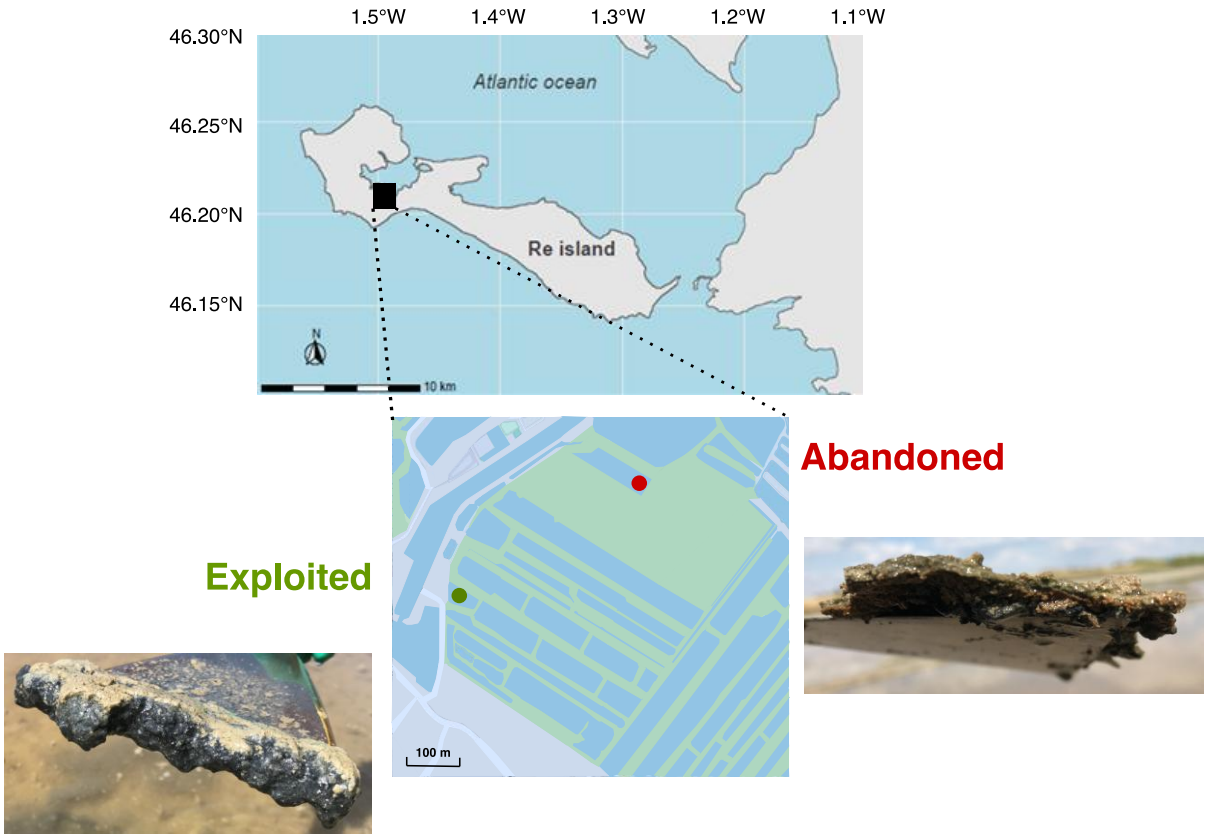
Figure 3: Bacterial community compositions at the phylum level in the abandoned and exploited sites. The bar plots represent each replicate, presenting the phyla with a relative abundance above 1%. The class level within Proteobacteria phylum is shown.

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

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

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

723 Figure 1



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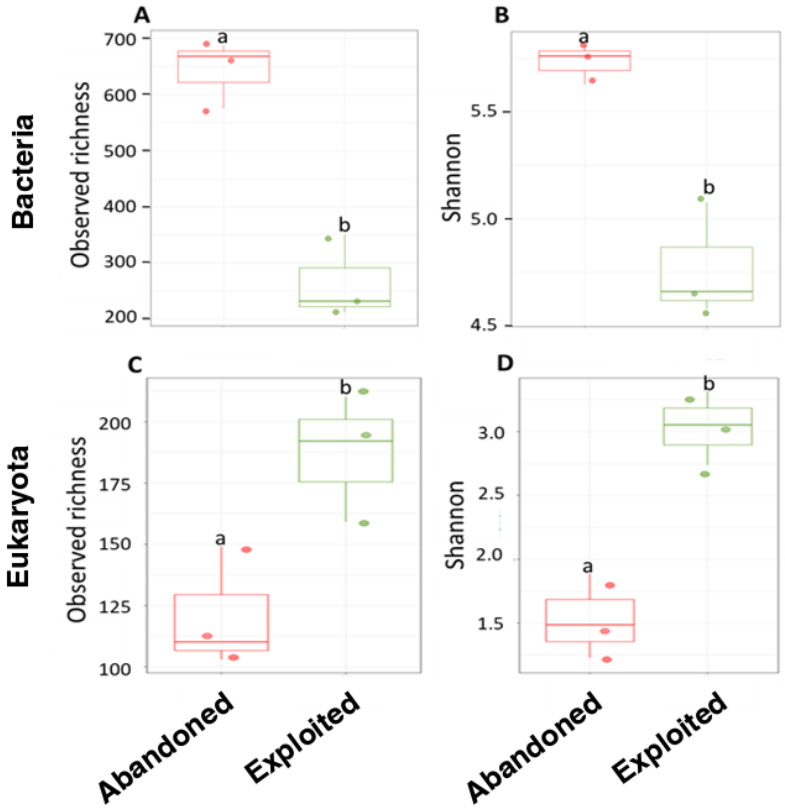
726 Figure 2



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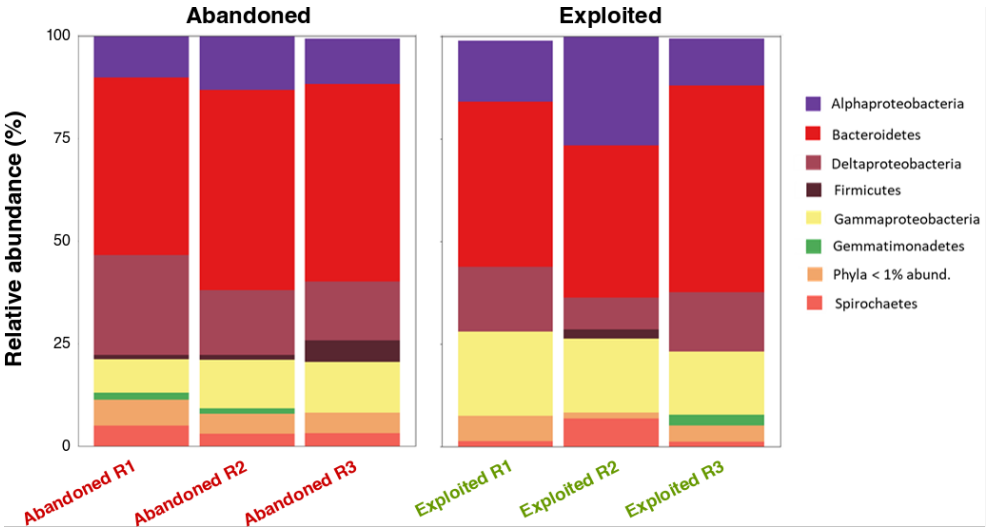

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732 Figure 4

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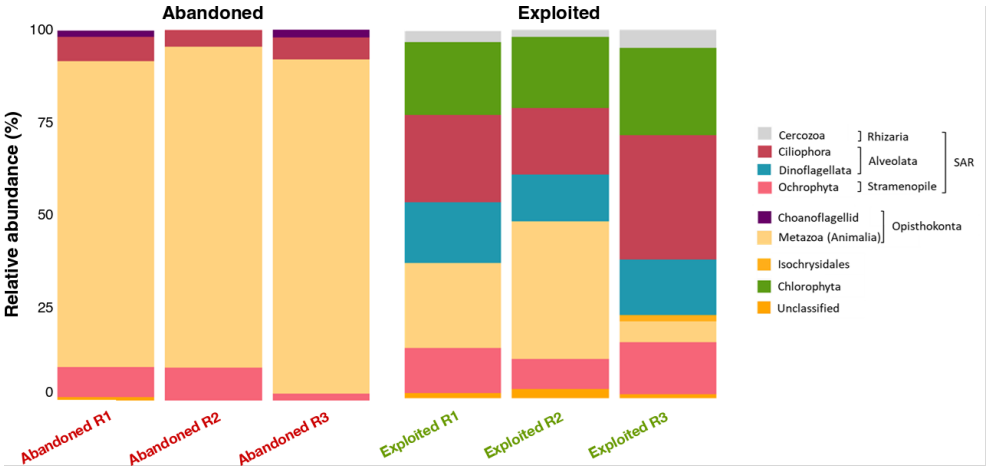

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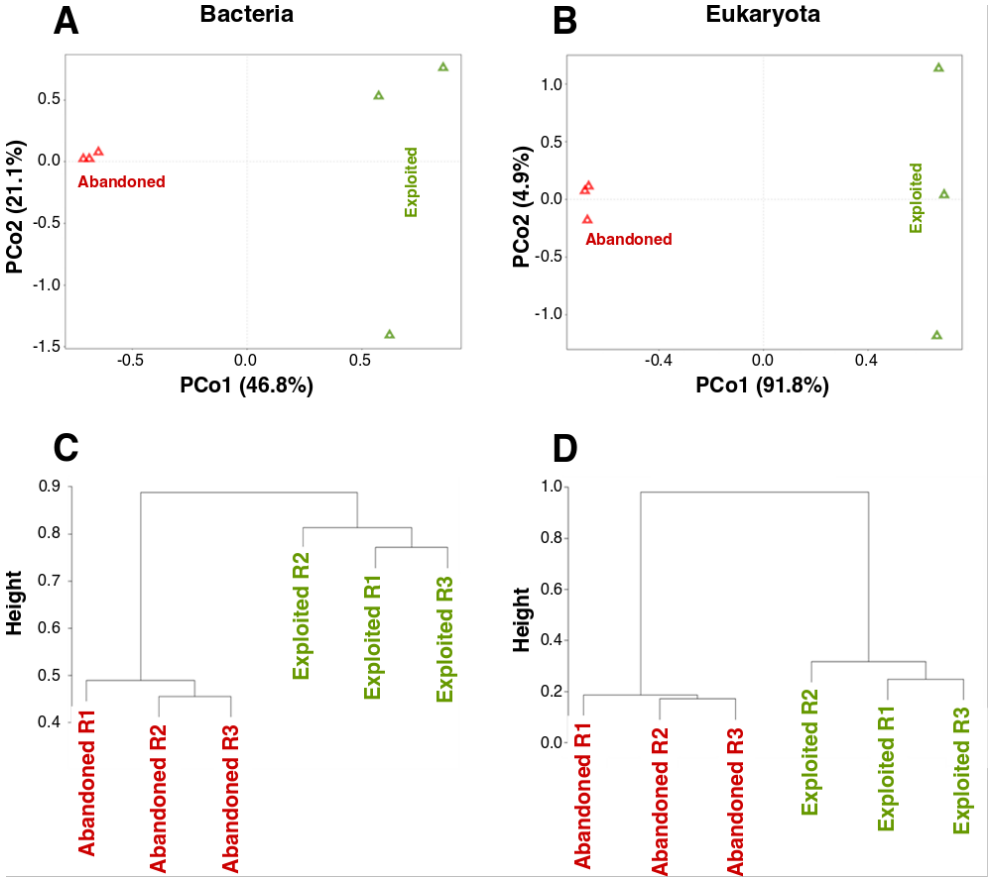
736 Figure 5



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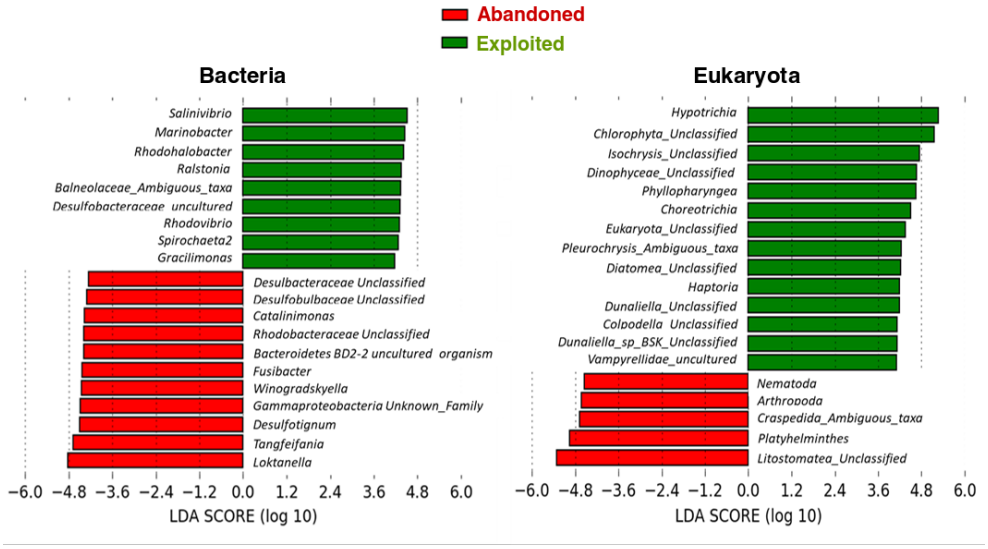
739 Figure 6



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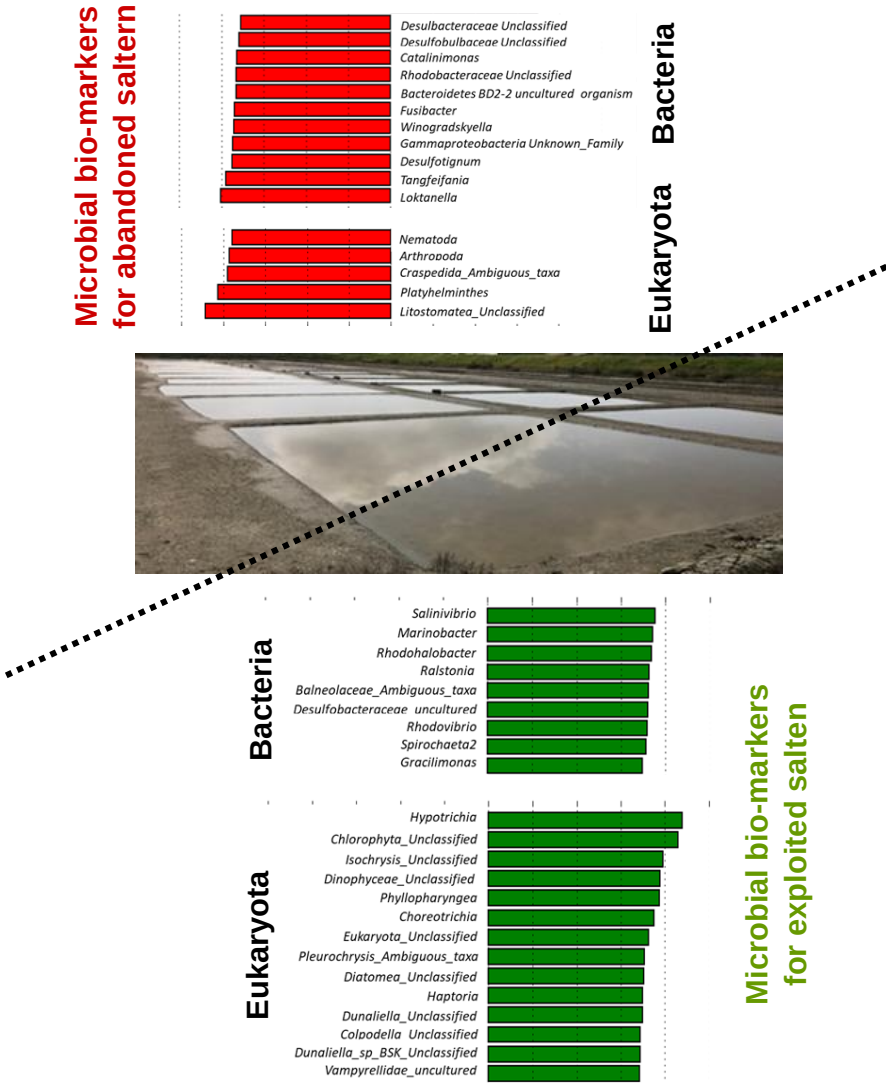
742 Graphical abstract



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