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Bacterial community assemblages in sediments under high anthropogenic pressure at Ichkeul Lake / Bizerte Lagoon hydrological system, Tunisia

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Highlights

- Evidence of bacterial continuum in Ichkeul Lake / Bizerte Lagoon
- The lake and the lagoon have specific bacterial assemblages
- Each type of pollutant is associated with specific bacterial genera
- Uncontaminated sediments have also specific bacterial genera
- Potential to develop tools for managing bacterial resources

Abstract

Bacterial communities inhabiting sediments in coastal areas endure the effect of strong anthropogenic pressure characterized by the presence of multiple contaminants. Understanding the effect of pollutants on the organization of bacterial communities is of paramount importance in order to unravel bacterial assemblages colonizing specific ecological niches. Here, chemical and molecular approaches were combined to investigate the bacterial communities inhabiting the sediments of the Ichkeul Lake / Bizerte Lagoon, a hydrological system under anthropogenic pressure. Although the microbial community of the Ichkeul Lake sediment was different to that of the Bizerte Lagoon, common bacterial genera were identified suggesting a lake-lagoon continuum probably due to the hydrology of the system exchanging waters according to the season. These genera represent bacterial "generalists" maintaining probably general biogeochemical functions. Linear discriminant analysis effect size (LEfSe) showed significant differential abundance distribution of bacterial genera according to the habitat, the pollution type and level. Further, correlation analyses identified specific bacterial genera which abundance was linked with pesticides concentrations in the lake, while in the lagoon the abundance of specific bacterial genera was found linked with the concentrations of PAHs (Polycyclic aromatic hydrocarbons) and organic forms of Sn. As well, bacterial genera which abundance was not correlated with the concentrations of pollutants were identified in both lake and lagoon. These findings represent valuable information, pointing out specific bacterial genera associated with pollutants, which represent assets for developing bacterial tools for the implementation, the management, and monitoring of bioremediation processes to mitigate the effect of pollutants in aquatic ecosystems.

Keywords: 16S rRNA sequencing, pollution, pesticide, PAH, network analysis

1. Introduction

Lagoons and lakes are highly productive ecosystems, providing important ecological services such as breeding grounds and nurseries for many fish and bird species (Vasconcelos et al., 2011). They are threatened by human activities, and therefore they need careful monitoring because they are very vulnerable (Pérez-Domínguez et al., 2012). Their status may change rapidly in response to natural- and human-driven events (Cravo-Laureau et al., 2017a; Duran et al., 2015c). Sediments are complex habitats densely colonized by diverse microorganisms (Brito et al., 2009; Chronopoulou et al., 2013; Duran et al., 2015a; Fourcans et al., 2008). In sediments, microbial communities are involved in the decomposition of organic matter (Schultz and Urban, 2008). Microorganisms play a key role in carbon, nitrogen and phosphorus cycling (Andrews et al., 2011) as well as in determining the fate of pollutants (Bordenave et al., 2004a; Bordenave et al., 2004b; Guermouche M'rassi et al., 2015; Pringault et al., 2008; Stauffert et al., 2013). Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are considered as persistent organic pollutants (POPs), which are ubiquitous in aquatic ecosystems (Doong et al., 2008; Kjellerup et al., 2014; Mwanamoki et al., 2014). POPs are mainly derived from industrial and agricultural sources, as well as maritime traffic (Chang and Doong, 2006; Wen et al., 1994). They enter aquatic environments through atmospheric deposition and run-off (Botta et al., 2009; Duran and Cravo-Laureau, 2016). Their fate in the marine environment depends on abiotic and biotic factors (for review, see Duran and Cravo-Laureau, 2016). The rapid development of molecular methods for microbial ecology has provided detailed information on microbial diversity and dynamics in marine sediments (Bordenave et al., 2008; Stauffert et al., 2014; Terrisse et al., 2017; Thiyagarajan et al., 2010). The molecular methods, particularly high-throughput sequencing technologies have been proved to be useful tools for bio-monitoring aquatic ecosystem (Cravo-Laureau and Duran, 2014; Cravo-Laureau et al.,

2017b). Their utilization in combination with chemical analyses might shed light on the ecological role and the adaptation of bacteria to environmental factors. Correlation analyses allowed the exploration of the interactions between microorganisms and their habitat (Barberan et al., 2012; Fuhrman and Steele, 2008; Llado et al., 2015; Williams et al., 2014; Yergeau et al., 2012). Particularly, such analyses have identified possible pollutant degraders (Llado et al., 2015; Yergeau et al., 2012), thereby obtaining valuable information for guiding the management of microbial communities to mitigate the effect of pollutants.

The Ichkeul Lake / Bizerte Lagoon hydrological system (Tunisia) is located in an area under high anthropic pressure. The Ichkeul Lake, Ramsar protected area, is important for migrating birds (Ramdani et al., 2001). The contaminants entering into the Ichkeul Lake / Bizerte Lagoon hydrological system (Stevenson and Battarbee, 1991; Trabelsi and Driss, 2005) have been shown to affect aquatic organisms and migratory birds (Ben Said et al., 2009; Casagranda and Boudouresque, 2010; Gharbi-Bouraoui et al., 2008). During the past decades several projects have studied the PAHs in the sediment of Bizerte Lagoon and their impact on the benthic microorganisms and organisms (Ben Said et al., 2009; Ben Said et al., 2008; Ben Said et al., 2015; Louati et al., 2013a; Louati et al., 2013b; Trabelsi and Driss, 2005). Recently, we characterized the contamination of the Ichkeul Lake / Bizerte Lagoon hydrological system. Almost all stations exceeded the threshold effect level (TEL) of the standards published by NOAA (Ben Salem et al., 2017). The Bizerte Lagoon has been shown to contain industrial contaminants including hydrocarbon (PAHs, polycyclic aromatic hydrocarbons) and metallic compounds, while the Ichkeul Lake was found to contain agrochemicals contaminants including organochlorine pesticides such as endrin and dieldrin (Ben Salem et al., 2016a; Ben Salem et al., 2016b; Ben Salem et al., 2017). In a previous study, based on 16S rRNA gene T-RFLP (terminal restriction fragment length polymorphism) analysis investigating the bacterial communities in four contrasted stations of the Ichkeul

Lake / Bizerte Lagoon hydrological system (two located at the Ichkeul Lake, and two at the Bizerte Lagoon), we demonstrated that bacterial community compositions were linked with the type and level of pesticide contamination at the dry season (Ben Salem et al., 2016a). The current study aims to characterize the bacterial communities from the sediments of the Ichkeul Lake / Bizerte Lagoon hydrological system. We further characterize the bacterial communities structure by 16S rRNA genes T-RFLP analysis, expanding the study by investigating 20 stations around the Ichkeul Lake / Bizerte Lagoon hydrological system at two contrasted seasons (April, dry season; September, wet season). Also, we further characterize the bacterial composition of four contrasted stations by sequencing 16S rRNA gene PCR-amplified fragments via 454-pyrosequencing. The effect of organic contaminants such as PAHs, PCBs, OCPs on the bacterial assemblages were determined. Specific bacteria, which abundance was linked with the presence of a pollutant, were identified.

2. Materials and Methods

2.1. Site description

The Ichkeul Lake / Bizerte Lagoon hydrological system, North Tunisia, suffers the effects of human activities because it is located in an area under high anthropic pressure. Bizerte Lagoon has a central role because connected to the Mediterranean Sea and to the Ichkeul Lake via the Tinja River; the hydrological system receives water via different rivers (Oueds) all around (Fig. 1). The lake experiences large fluctuations in salinity each year, from freshwater with average salinity around 4 PSU (Practical salinity unit) in winter (wet season) due to rivers flood, to saline ecosystem with average salinity around 35 PSU in summer (dry season) receiving seawater from the Bizerte Lagoon and because of evaporation. In a previous study,

130 we demonstrated that pollution levels fluctuated according to seasons (Ben Salem et al.,
131 2016a).

132 **2.2. Sampling**

133 Sediment samples were collected from 20 stations (ST) located all around the Ichkeul Lake /
134 Bizerte Lagoon hydrological system (Fig. 1): ST1 to ST8 in the lake and ST11 to ST20 in the
135 lagoon, ST9 on the channel connecting the lagoon to the sea, and ST10 in the Mediterranean
136 Sea. The lake and lagoon stations were located at the river mouths (13 stations), close to
137 industrial and agricultural areas (4 stations), and at the centers of the lake and the lagoon (2
138 stations). Two sampling campaigns were performed: September 2011 (wet season) and April
139 2012 (dry season). Surface sediments (0-10 cm) were collected in triplicates (3 independent
140 samples, i.e. biological replicates) at each station with a Van Veen grab as previously
141 described (Ben Salem et al., 2016a). Collected sediments were dispatched in cryotubes (3
142 technical replicates were taken), quickly frozen in liquid nitrogen for microbial analyses, and
143 in glass bottles kept at 4°C for chemical analyses. At lab, samples for microbial analyses were
144 stored at -80°C and those for chemical analyses at -20°C until use.

145 **2.3. Physical-chemical analyses**

146 Physical-chemical parameters, including temperature, dissolved oxygen, pH, salinity,
147 phosphate, ammonia, nitrite, and nitrate were determined as previously described (Ben Salem
148 et al., 2016a). Chemical analyses for contaminants followed the QuEChERS procedure
149 described in Ben Salem et al. (2016b). Chemical data and physical-chemical parameters are
150 summarized in supplementary material (Table S1). All chemical data and physical-chemical
151 parameters are presented in details in Ben Salem et al. (2017).
152 The contamination contents of the sediments were compared to sediment quality guidelines
153 (SQG) in order to determine their potential toxic effect. Sediments were classified according
154 the quality criteria developed by Bakke et al. (2010) for PCBs, OCPs, and PAHs. The toxic

potential of the sediments was evaluated by calculating the mean effect range medium (ERM) quotient (m-ERM-Q) as previously described (Long et al., 1998).

2.4. DNA extraction, PCR and T-RFLP analyses

DNA extractions were performed using 250 mg of frozen sediment with the UltraClean soil DNA isolation Kit (MoBio Laboratories, CA) as previously described (Precigou et al., 2004). The bacterial universal primers 1387R 5'-GGGCGGWGTGTACAAGGC-3' and 63F 5'-CAGGCCTAACACATGCAAGTC-3' (Marchesi et al., 1998) were used for 16S rRNA gene amplifications. Primers were fluorescently labeled for T-RFLP analysis, which was performed as previously described (Precigou et al., 2001) using 10 ng of extracted DNA. PCR mixture and conditions were performed as previously described (Vercraene-Eairmal et al., 2010). PCR amplicon purification, digestion and electrophoresis separation have been previously described (Vitte et al., 2011). T-RFLP profiles were analyzed as previously described (Cravo-Laureau et al., 2011).

2.5. Sequencing and data analysis

Among the 20 stations, 4 stations were selected according to the environmental parameters for 454 pyrosequencing analyses. The analysis was performed with the samples collected at the dry season (April 2012) because the highest levels of contaminants (particularly PAHs and pesticides) were recorded (supplementary material Table S1), and also because important contrasted conditions were detected between Ichkeul Lake and Bizerte Lagoon (Table 1, and supplementary material, Table S1). Two stations from the Ichkeul Lake (salinity 2.6 ± 0, pH 8.29 ± 0.02; supplementary material Table S1) were chosen for contrasted contaminations: ST5, showing multiple contaminations by PAHs and pesticides; and ST8, the most contaminated by OCPs (Table 1; supplementary material, Table S1). Similarly, two stations from the Bizerte Lagoon (salinity 29.5 ± 0.4, pH 8.37 ± 0.03) were chosen for contrasted

contaminations: ST12, the most contaminated by PAHs; and ST17, the less contaminated of the entire complex (Table 1; supplementary material, Table S1). Three replicates for each station were sequenced at Molecular Research LP DNA (MR DNA sequencing service, Shallowater, TX, USA) using Roche 454 FLX titanium as previously described (Dowd et al., 2008; Duran et al., 2015a).

QIIME (Quantitative Insights Into Microbial Ecology; Caporaso et al., 2010) was used to analyse the 16S rRNA gene sequence reads as described (Terrisse et al., 2017). Briefly, data cleaning was performed according to Quince et al. (2009) by denoising of the data and by eliminating chimera and sequences with length under 450 bp, with mistakes in primers sequences or homopolymers. OTU picking was performed following the Usearch method (Edgar, 2010) defining reference sequences by applying a 97% similarity threshold. The taxonomic assignment was performed with BLASTn against May 2013 Greengenes database (DeSantis et al., 2006; McDonald et al., 2012) to establish the OTU table. The OTU abundances for each sample were rarefied to the minimum read number observed (12,977 sequences) for a sample using the R package vegan (Oksanen et al., 2013; Version: 2.0–10). The complete dataset was deposited in the NCBI Sequence Read Archive (SRA) database (SUB5043721). It is available under the Bioproject ID PRJNA515186.

2.6. Statistical analysis

Canonical Correspondence Analysis (CCA) correlating bacterial community structure (T-RFLP patterns) with chemical data (PAH and pesticide concentrations) and physical-chemical parameters, was performed with MVSP v3.22 software (Kovach Computing Service, Anglesey Wales). T-RFLP profiles were normalized by calculating relative abundances of each TRF, as previously described (Dominique et al., 2007). Data were square root transformed to get a normal distribution of the data (Dollhopf et al., 2001).

All statistical analyses based on sequencing data were performed on the random subsample of 12,977 bacterial sequences, corresponding to the smaller number of sequences per sample in the dataset. Alpha diversity indices (Shannon-Wiener H' and richness R), based on rarefied OTU table, were calculated using PRIMER v5.0 (Plymouth Routines in Multivariate Ecological Research). The overall differences of these indices between the stations were tested by ANOVA and Tukey HSD multiple comparisons (pairwise comparisons). Significant difference was assumed when $p < 0.05$. The contribution of individual OTUs explaining the differences between stations was determined by SIMPER (similarity percentages) based on Bray-Curtis distance measure.

Linear discriminant analysis effect size (LEfSe; Segata et al., 2011) was performed to determine biomarkers of contamination and habitat as previously described (Jeanbille et al., 2016b). Briefly, 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 LDA threshold score of 2.0 and 1000 bootstrap interations were applied. Heatmap and clustered correlation (Weinstein et al., 1997) were performed with CIMminer (http://discover.nci.nih.gov/cimminer/). These analyses were performed at the genus level derived from the OTU abundance table in order to determine the correlations between bacterial genera abundances and pollutant concentrations. Correlation analysis was based on Pearson correlation. The hierarchical clustering of correlations was based on ward method. Strong correlations (>|0.5|) were considered. The distribution of OTUs identified in the bacterial patterns of the different stations was visualized with Venn diagrams constructed with Venny using OTU occurrence table as previously described (Duran et al., 2015a). OTUs present at least in two of the triplicates were retained for the analysis.

3. Results and discussions

3.1. Contamination level

The chemical analyses of the sediments collected during September 2011 and April 2012 sampling campaigns (supplementary material, Table S1) have been presented in detail in Ben Salem et al. (2017). Table 1 summarizes the chemical data showing the sum of the different types of pollutants obtained in April 2012 for four representative and contrasted stations: 2 from the lake (ST5 and ST8) and 2 from the lagoon (ST12 and ST17). The Bizerte Lagoon was characterized by PAH concentrations ranging from 137 ± 26 to 17988 ± 1183 ng g^{-1}, corresponding to class I (ST12, insignificantly polluted) and class IV (ST17, severely polluted), respectively according to the classification developed by Bakke et al. (2010). Both stations of the Ichkeul Lake (ST5 and ST8) were classified as class II with PAH concentrations of 1362 ± 560 and 444 ± 24 ng g^{-1}, respectively. Considering the contamination by organochlorine pesticides (OCPs), the Ichkeul Lake stations were classified as class IV (severely polluted) while the Bizerte Lagoon stations were classified as class III (markedly polluted) according to the Bakke et al. (2010) classification. The OCPs, including endrin, dieldrin and lindane, and their derivatives, were detected in the Ichkeul Lake at concentrations ranging from 160 ± 14 to 2021 ± 85 ng g^{-1}. Additionally, the chemical analyses revealed low PCB contamination of the sediments (ranging from 2.48 ± 0.8 to 5.58 ± 1 ng g^{-1}). The sediments were classified as class I (insignificantly polluted) except for the station ST8 that was classified as class II (moderately polluted). In order to take into account the multiple contaminations, the mean effect range medium quotients (m-ERM-Q) were calculated (Table 1; supplementary material, Table S1). The m-ERM-Q showed that the toxic potential of the sediments was higher (p value <0.01) in Ichkeul Lake stations than in Bizerte Lagoon stations at both seasons (supplementary material, Table S1). The station were classified according to increasing m-ERM-Q potential toxicity of sediments as follow ST17

253 < ST12 < ST5 < ST8 (Table 1). Station ST17 was considered as lowly contaminated, while
254 stations ST5, ST8 and ST12 were considered as highly contaminated.
255 The chemical data and physical-chemical parameters (supplementary material, Table S1)
256 presented in detail in Ben Salem et al. (2017) were used in the present study to characterize
257 the bacterial community structure according to environmental parameters.

258 **3.2. Bacterial community structure**

259 Correspondence canonical analysis (CCA) using T-RFLP patterns and chemical data
260 separated bacterial communities of the two ecosystems, lake and lagoon irrespective of the
261 season (Fig. 2a). Regarding the environmental parameters, the salinity, the temperature, and
262 the concentrations of nitrite and ammonia were the main factors influencing the bacterial
263 community composition (based on T-RF abundances) of the lagoon, while the bacterial
264 community composition of the lake correlated with dissolved oxygen (DO) and phosphate
265 concentrations. This observation was consistent with previous studies showing that bacterial
266 communities are affected by salinity (Jeanbille et al., 2016a; Jeanbille et al., 2016b; Xi et al.,
267 2014) and nutrients (Bodoque et al., 2017; Liu et al., 2015; Yao et al., 2017). Particularly,
268 nitrite and ammonia concentrations may affect the bacterial populations involved in the
269 nitrogen cycle in lagoon sediments as shown for saline lake sediments (Lipsewers et al., 2016)
270 and marine contaminated sediments (Mahmoudi et al., 2015). The bacterial populations
271 involved in the nitrogen cycle have been shown to play an important role in hydrocarbon
272 removal in marine sediments (Mahmoudi et al., 2015; Stauffert et al., 2016). The CCA also
273 showed that the pH and the concentrations of nitrate and organo-tin compounds influenced
274 slightly the bacterial community compositions probably because they varied within a small
275 range in the studied sediments.
276 Regarding the contaminants, the CCA analysis showed that the bacterial community
277 composition was correlated with pesticide concentrations in the lake, while bacterial

278 community composition was linked with PAHs concentrations in the lagoon (Fig. 2a). These
279 observations confirmed those previously reported (Ben Said et al., 2009) that showed the
280 impact of PAHs on bacteria communities in Bizerte Lagoon sediments. Considering the lake,
281 the bacterial community composition was correlated with PAHs concentrations in April,
282 except for those of station ST8 that was correlated with PCBs and Pesticides concentrations,
283 while bacterial community composition was correlated with the concentration of organo-tin
284 compounds in September (Fig. 2b). This observation suggested seasonal fluctuations in the
285 input of contaminants. It is likely that the lake receives pesticide inputs from crop treatments
286 in April. Additionally, the hydrology of the ecosystem might also play a crucial role: during
287 the dry season (April) lagoon water contaminated by PAHs enters into the lake (Ben Salem et
288 al., 2017). Considering the lagoon, the bacterial community composition was correlated with
289 PAHs and PCBs concentrations in April (Fig. 2c), suggesting that the pollutants were
290 concentrated (concentrating effect) during the dry season. It is likely that the dry season
291 represent a critical season for the Ichkeul Lake / Bizerte Lagoon hydrological system because
292 the environmental conditions favor the concentrating effect of pollutants in both the lake and
293 the lagoon. It is thus of paramount importance to investigate how benthic bacterial
294 communities are affected by such inputs of pollutants. The sediments from the stations ST5
295 and ST8 (lake) and the stations ST12 and ST17 (lagoon) collected in April were retained for
296 further bacterial characterization because they presented different type of contamination while
297 slightly influenced by environmental parameters (Fig. 2b,c; Table 1).

298 **3.3. Bacterial community composition**

299 In order to further investigate the effect of pollutants on bacterial communities at the dry
300 season (April), the composition of bacterial communities was determined by high throughput
301 pyrosequencing. Four stations showing contrasted chemical characteristics were chosen
302 (Table 1): station ST17 (lagoon), the less contaminated; station ST12 (lagoon), the most

303 contaminated with PAHs; station ST8 (lake), the most contaminated with OCPs (pesticides);
304 and station ST5 (lake), contaminated with PAHs and OCPs mixture. Three replicates from
305 each station were analysed. After trimming, the 191,278 sequences (400 bp) retained (12,977
306 sequences per sample after resampling) were dispatched within 858 OTUs, which were
307 grouped into 44 different phyla (Fig. 3). Rarefaction curves (Supplementary data Fig. S1)
308 showed high sequencing coverage in all samples. The coverage (~ 94%) indicated that the
309 sequencing effort was sufficient to assess the bacterial community diversity (Table 2). The
310 sequence affiliation showed that Proteobacteria largely dominated the sediments, with
311 predominance of Deltaproteobacteria (24%), Gammaproteobacteria (23%) and
312 Alphaproteobacteria (9%); Epsilonproteobacteria and Betaproteobacteria accounting for 6%
313 and 5% respectively. The dominance of Proteobacteria, particularly Deltaproteobacteria and
314 Gammaproteobacteria, has been often reported in both lake and lagoon sediments (Borin et
315 al., 2009; Obi et al., 2016; Pavloudi et al., 2016; Quero et al., 2015; Sun et al., 2013)
316 suggesting that they have a pivotal role in biogeochemical cycles in sediment environments.
317 Besides Proteobacteria, Bacteroidetes (9%), Chloroflexi (6%), Planctomycetes (2%) and
318 Nitrospirae (2%) were the major phyla detected in all four stations (Fig. 3), which was
319 consistent with previous observation in sediment environments (Behera et al., 2017; Fan et al.,
320 2016). These phyla have been detected in freshwater and marine sediments (Behera et al.,
321 2017; Fan et al., 2016; Jacquiod et al., 2018b; Paissé et al., 2012; Pavloudi et al., 2016).
322 Bacteroidetes are well known for their capacity to degrade organic matter (Hunter et al., 2006;
323 Zeng et al., 2011). They were in high abundance in contaminated sediments (Paisse et al.,
324 2008; Quero et al., 2015; Sun et al., 2013). Chlorofexi have been found as main players in the
325 transformation of organic compounds including organo-chlorines such as PCBs and pesticides
326 (Fagervold et al., 2007; Hug et al., 2013; Matturro et al., 2016a). Planctomycetes, which have
327 the capability to oxidize ammonia aerobically and anaerobically (Junier et al., 2010), have

been widely detected in contaminated sediments (Dell'Anno et al., 2012; Quero et al., 2015). Similarly, Nitrospirae members playing an important role in the nitrogen cycle (Daims, 2014) have been detected in contaminated sediments by organic compounds including pesticides and PAHs (Ding et al., 2017; Fernanda Sánchez-Soto Jiménez et al., 2018; Stauffert et al., 2013). Alpha diversity indices were not significantly different between stations (Table 2), an observation that was in contradiction with other studies showing that the presence of pollutants either reduce (Haller et al., 2011; Johnston and Roberts, 2009) or increase (Duran et al., 2015a; Jacquiod et al., 2018a) bacterial diversity. It was thus surprising to observe similar bacterial diversity indexes in sediments presenting different pollutant types and levels. Similar observations have been reported by comparing uncontaminated and contaminated sediments (Paisse et al., 2008; Yin et al., 2015) and soils (Berg et al., 2012). The pollution history, particularly the fluctuant and successive pollution loads resulting in legacy effects, may explain the fact that bacterial diversity was maintained (Jurburg et al., 2017; Nunes et al., 2018). Also, the coalescence of bacterial communities, mixing bacterial communities from different ecosystems (Mansour et al., 2018; Rillig et al., 2015), here lake and lagoon, may contribute to attenuate the diversity differences between the ecosystem, as previously described in river sediments (Yin et al., 2015). The Venn diagram, showing shared OTUs between lake and lagoon stations (Fig. 4a) evidenced the ecological continuum between the lake and the lagoon, which are closely linked via the Tinja River (Fig. 1). Although similar diversity indexes were observed, the composition of the bacterial diversity was different. Indeed, the Venn diagram revealed that each station was characterized by specific OTUs, only 211 OTUs being shared between the stations (Fig. 4a). Such observation was consistent with previous reports on bacterial communities in coastal areas (Duran et al., 2015b; Ikenaga et al., 2010; Misson et al., 2016), indicating that bacterial communities were structured according to the environmental conditions, as revealed by the T-RFLP analyses (Fig. 2). Additionally,

linear discriminant analysis effect size (LEfSe) detected taxa differentially abundant according the ecosystems. LEfSe showed the habitat specificity of genera that were specifically more abundant in either the lake or the lagoon (Fig. 4b). This observation was consistent with previous reports showing habitat specificity for microbial taxa (Jeanbille et al., 2016a; Jeanbille et al., 2016b; Xi et al., 2014). Similarly, LEfSe revealed the presence of specific genera (*Sulfurimonas*, *Acidovorax*, *Pelobacter*, *Sediminibacter*, *Sinobacteraceae*, *Desulfobulbus*, and *Sulfurovum*) in the contaminated stations (Fig. 4c). These observations were further supported by clustering and SIMPER analyses. Clustering analysis, based on Pearson correlation, showed that the lake stations had bacterial communities with different composition to those of the lagoon stations (Fig. 5), which was corroborated by SIMPER analysis (50%). The bacterial communities from the lake stations ST5 and ST8 showed more similar composition (SIMPER: 93%) than those of the lagoon stations ST12 and ST17 (SIMPER: 71%). Consistent with LEfSe, the less contaminated station ST17 exhibited specific dominant OTUs affiliated to *Rhodobacter*, *Planctomycetaceae*, *Rhizobium*, *Rhodopirellula*, *Blastopirellula*, *Nitrosococcus* and *Sorangium* genera (Fig. 5), which related members are typically detected in marine ecosystems (Ben Said et al., 2008; Brinkhoff et al., 2012; Hernandez-Raquet et al., 2006; Jasmin et al., 2017; Klotz et al., 2006). Regarding the contaminated stations, the clustering analysis allowed the identification of specific OTUs for each station. The most PAH-contaminated station ST12 was dominated by specific OTUs belonging to *Sediminibacter* and *Loktanella* genera and other genera affiliated to *Rhodobacteraceae*, *Flavobacteriaceae* and *Saprospiraceae* families (Fig. 5), which related members have been detected in marine sediments after oil spill (Acosta-Gonzalez et al., 2015; Galazka et al., 2018; Jimenez et al., 2007). The lake stations ST5 and ST8 were dominated by specific OTUs related to *Thiobacillus*, *Nitrospira* and *Acidovorax* genera, *Escherichia-Shigella* group, and other genera affiliated to *Gemmatimonadaceae*, *Nitrospiraceae*,

Caldilineaceae and *Desulfarculaceae* families (Fig. 5), which was consistent with LEfSe (Fig. 4). Related members of these taxa have been found in sediments contaminated by hydrocarbons (Jochum et al., 2018; Patel et al., 2015; Reyes-Sosa et al., 2018) and pesticides (Douglass et al., 2015; Jiang et al., 2018).

Although LEfSe revealed that the abundance of some taxa were different between the lake and lagoon ecosystems (Fig. 4), the clustering analysis showed that the four stations shared dominant OTUs affiliated to *Acidithiobacilus*, *Desulfobulbus*, *Sinobacteraceae*, *Robiginitalea*, *Anaerolineaceae* and *Nannocystineae* (Fig. 5). Members of the *Acidithiobacillus* genera have been detected in metals contaminated ecosystems (Giloteaux et al., 2010), particularly in sediments (Fonti et al., 2013). It is not surprising to detect metal-resistant bacteria in the Ichkeul Lake / Bizerte Lagoon hydrological system since high levels of metal contents have been detected in the sediments (Ben Said et al., 2009; Zaaboub et al., 2015). The detection of members related to *Desulfobulbus*, *Robiginitalea*, *Sinobacteraceae, Anaerolineaceae* and *Nannocystineae* encompassing marine species, further support the ecological continuum between the Ichkeul Lake and Bizerte Lagoon. Among them some have been found in contaminated sediments bearing the capacity to degrade hydrocarbons (Li et al., 2017; Yan et al., 2018), and to resist and transform metals (Meng et al., 2019; Wang et al., 2018).

3.4. Influence of contamination on bacterial assemblages

In order to further decipher the associations between bacterial genera and environmental parameters clustered correlation (ClusCor) analysis was performed at the genus level derived from the OTU abundance table (Fig. 6). First, sequences related to 11 genera including *Sulfurovum*, *Pseudomonas, Leptospirillum, Sulfurimonas*, *Acidovorax*, and *Nitrospira* genera, and genera related to *Anaerolineaceae*, *Desulfarculaceae, Caldilineaceae*, *Gemmatimonadaceae*, and *Nitrospiraceae* compose the Group1a. The abundance of the Group1a genera showed strong (0.6>Pearson correlation<0.8) to very strong (Pearson

403 correlation>0.8) positive correlations with the concentration of the different pesticides
404 (PCBs, Lindane, and other OCPs), and strong (-0.6>Pearson correlation>-0.8) to very strong
405 (Pearson correlation<-0.8) negative correlation (exclusion) with PAHs concentrations. This
406 Group1a was closely related with the Group1b including *Desulfobulbus* and *Thiobacillus*
407 genera, and genera related to *Nannocystineae*, *Sinobacteraceae*, and *Escherichia-Shigella*,
408 which abundances showed strong (0.6>Pearson correlation<0.8) to very strong (Pearson
409 correlation>0.8) positive correlations with the concentrations of pesticide compounds and
410 strong negative correlation (-0.6>Pearson correlation>-0.8, i.e. exclusion) with the other
411 pollutants. Consistently, LEfSe identified all these genera (Group1a and Group1b)
412 specifically abundant for either the lake or the contamination (Fig. 4). Several *Pseudomonas*
413 and *Acidovorax* species have been isolated for their ability to degrade pesticides and other
414 organic compounds in diverse habitats (Marican and Duran-Lara, 2018; Song et al., 2000).
415 Interestingly, the presence of the sulfur-oxidizing bacteria related to the *Sulfurovum* and
416 *Sulfurimonas* genera suggested the potential anaerobic degradation of pesticides since these
417 genera have been described for their capacity to perform reductive dechlorination of PCB in
418 marine sediments (Matturro et al., 2016b), often associated with sulfate-reducing bacteria
419 (SRB). Also, both genera, *Sulfurovum* and *Sulfurimonas,* have been shown to be able to grow
420 under microaerophilic conditions using oxygen or nitrate as electron acceptor (Mitchell et al.,
421 2014; Xu et al., 2017). It is likely that these genera in association with the SRB
422 *Desulfarculaceae* related genera and *Desulfobulbus* in the Group1 assemblage might play a
423 role in the sulfur cycle. They probably use the hydrogen sulfur (H_2S) produced by SRB and
424 oxygen for their growth playing a role in the degradation of pesticides. The genera related to
425 the *Nitrospiraceae* have been found dominant in contaminated aquatic ecosystems:
426 *Nitrospira* dominant in organic matter contaminated waters (Huang et al., 2017; Wang et al.,
427 2016), while *Leptospirillum* in the presence of metals and metalloids (Wang et al., 2013).

Also, the presence of *Nitrospira* together with *Thiobacillus, Gemmatimonadaceae,* and the genera of the phylum Chloroflexi (*Anaerolineaceae* and *Caldilineaceae*), denitrifying genera, suggested a contamination from wastewater treatment systems as previously reported (Aanderud et al., 2018; Zhang et al., 2017), and further supported by the presence of *Escherichia-Shigella* and *Sinobacteraceae* related genera (Zhang et al., 2018). Related members of the *Nannocystineae* genera have been described harbouring the capacity to degrade complex macromolecules (Garcia and Müller, 2013). They have been detected in polluted sediments (Acosta-González et al., 2013), but their potential for pollutant degradation still to be demonstrated.

The Group2 included the genera related to *Flavobacteriaceae*, *Flammeovirgaceae, Saprospiraceae, Rhodobacteraceae* families, and the genera *Actibacter*, *Sediminibacter*, *Robiginitalea*, *Loktanella*, *Pelobacter*, *Desulfuromonas*. The abundances of Group2 genera showed very strong positive correlation with the concentrations of PAHs and organic-SN compounds (Pearson correlation>0.8), and strong (-0.6>Pearson correlation>-0.8) to very strong (Pearson correlation <-0.8) negative correlations (exclusion) with the concentrations of lindane and other OCPs. These genera, which abundance was correlated to either the lagoon or the contamination level by LEfSe (Fig. 4), have been found in hydrocarbon contaminated marine sediments (Acosta-Gonzalez et al., 2015; Chen See et al., 2018; Galazka et al., 2018) and oil production facilities (Vigneron et al., 2016; Zapata-Peñasco et al., 2013). Particularly interesting, the presence of members related to the *Desulfuromonadaceae* family (*Pelobacter* and *Desulfuromonas)* in this Group2 assemblage that have been described in presence of pesticide and for their capacity of reductive dechlorination (Greene, 2014; Sercu et al., 2013). Such observation suggested that these genera might play a key role to cope with the multi-contamination in lagoon sediments.

Behind these two major groups, the ClusCor analyses identified bacterial assemblages (Group3) including genera, which abundances showed mainly strong (-0.6>Pearson correlation>-0.8) to very strong (Pearson correlation<-0.8) negative correlations (exclusion) with pollutant contents (Fig. 6). The Group3a included genera which abundance showed exclusion with PCBs and PAHs. The abundance of genera from Group3b showed exclusion with the content of all pesticides except heptachlor and strong positive correlation (0.6<Pearson correlation>0.8) with PAHs content (Fig.6). The Group3c correspond to genera which abundance showed exclusion with PCBs content, while the abundance of genera from Group3d showed exclusion with PCBs and lindane contents (Fig. 6). Noteworthy, most of the genera included in Group3c (*Candidatus-Thiobios*, *Rhizobium*, *Desulfocapsa*, *Planctomycetaceae*, *Rhodopirellula*, and *Blastopirellula*) showed exclusion with pollutants, which was consistent with LEfSe highlighting that the abundance of these genera was correlated with low contamination level (Fig. 4c). It is likely that these genera were sensitive to the presence of pollutants suggesting that their presence might report on ecosystem quality. However, further investigations are requested to understand their role in the ecosystem functioning.

The co-occurrence analyses showed specific bacterial assemblages linked with contaminants revealing possible metabolic capacities of bacterial genera. Our results are consistent with previous reports that revealed specific populations linked with pollutants in marine ecosystems (Duran et al., 2015a), in freshwater lakes (Warnecke et al., 2005), and in soils (Llado et al., 2015). Such information provides new insights useful to guide future research to answer important questions in the field of microbial ecology such as the role of specific populations in the ecosystem functioning and pollutants degradation, and the role of environmental parameters in driving bacterial assemblages.

4. Conclusions

The determination of bacterial composition in sediment of the Ichkeul Lake / Bizerte Lagoon hydrological system showed that the lake bacterial communities were distinct from those of the lagoon. Although the difference could be attributed to physical-chemical parameters, the salinity and the presence of pollutants playing an important role in structuring the bacterial communities, the study revealed that the lake and the lagoon shared some bacterial genera. Such result suggested a lake-lagoon continuum due to the hydrology of the system exchanging waters according to the season resulting in bacterial community coalescence. It is likely that such bacterial genera ensure universal biogeochemical functions and, therefore constitute "generalists" bacteria essential for the basic ecosystem functioning. The study also revealed "specialists" bacteria either for the habitat (lake or lagoon) or for the pollutants. Bacterial "specialists" able to cope with the presence of pesticides in the lake and bacterial "specialists" for PAHs and organic forms of Sn in the Lagoon. These findings represent valuable information, particularly the bacterial "specialists", which represent yet an unexplored metabolic potential for the development of bioremediation processes to mitigate the effect of pollutants in aquatic ecosystems. The study also revealed bacterial genera, which abundance showed exclusion (negative correlation), with the pollutants concentrations. They represent potential bacterial biomarkers reporting on ecosystem quality, which will be useful to develop bacterial tools for monitoring the effect of countermeasures taken for mitigating the pollution.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical statement

This article does not contain any studies with human participants or animals performed by any of the authors.

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965

966 **Figure legends**

967

968 **Figure 1. Map of the Ichkeul Lake / Bizerte Lagoon hydrological system.** The sampling
969 stations (ST, red) are indicated by numbers.

970

971 **Figure 2. Comparison of bacterial communities from stations of the Ichkeul Lake /**
972 **Bizerte Lagoon hydrological system.** The canonical correspondence analysis shows the
973 correlations between bacterial communities structures (T-RFLP patterns) and environmental
974 chemical data in a) the Bizerte Lagoon-Ichkeul Lake complex, b) in Ichkeul Lake sediments,
975 and c) in Bizerte Lagoon sediments. The analyses were performed in September 2011 (wet
976 season) and April 2012 (dry season). Lake September, pink circles; Lake April, blue circles;
977 Lagoon September, red diamonds; Lagoon April brown diamonds. The analyses are based on
978 biological triplicates for each station. ∑ Pesticides, total pesticides; ∑ PAHs, total PAHs; ∑
979 PCBs, total PCB; ∑ Sn, total Sn organic compounds; T°, temperature; m-ERM-Q, mean
980 effect range medium quotient; P, phosphate; DO, dissolved oxygen. The names of the stations
981 further characterized in this study are indicated ST5 and ST8 for the Ichkeul Lake, and ST12
982 and ST17 for the Bizerte Lagoon.
983

984 **Figure 3. Composition of bacterial communities from stations of the Ichkeul Lake (ST5**
985 **and ST8) and Bizerte Lagoon (ST12 and ST17) hydrological system.** The analysis was
986 performed at the phylum level applying a threshold similarity of 97 % for OTU identification.
987 The average of three replicates was used for the analysis. Other, correspond to phyla with
988 relative abundance < 5%.

989

990 **Figure 4. Comparison of bacterial communities from the different stations of the Ichkeul**
991 **Lake / Bizerte Lagoon hydrological system.** a) Venn diagram showing the number of shared
992 OTUs between stations as well as the specific OTUs for each station. b) Linear discriminant
993 analysis effect size (LEfSe) of the habitat (lake / lagoon). c) LEfSe analysis of the
994 contamination. LEfSe analysis shows the significant differential abundance distribution of
995 bacterial genera between the samples of environmental groups (lake / lagoon; high

contamination / low contamination). The analysis was performed applying a threshold similarity of 97 % for OTU identification. The contamination level (high / low) refers to mean effect range medium quotients (m-ERM-Q) presented in Table 1.

Figure 5. Heatmap comparing the bacterial communities from the different stations of the Ichkeul Lake / Bizerte Lagoon hydrological system. The heatmap with clustering analysis based on Pearson correlation shows the repartition of the 50 most abundant genera. The analysis was performed applying a threshold similarity of 97 % for OTU identification. The triplicates for each station are shown in the heatmap.

Figure 6. Clustered correlation (ClusCor) between bacterial OTUs and pollutants parameters. The analysis is based on Pearson correlation, red indicating high positive correlation and blue high negative correlation (p value <0.01). Clustering was performed applying Pearson correlations and Ward algorithm as cluster method. Abbreviations of pollutants are those indicated in Table 1. The analysis was performed at the genus level applying a threshold similarity of 97 % for OTU identification. The specific OTUs revealed by LEfSe analysis are coloured according to their specificity: lake (khaki), lagoon (red), high contamination (orange), and low contamination (blue).

Table 1: Chemical analyses of contaminants in the Ichkeul Lake / Bizerte Lagoon hydrological system and sediment toxicity potential. The data corresponds to the sum of the different types of pollutants obtained in April 2012 described in details by Ben Salem et al. (2017). Average ± SD are presented (n=3).

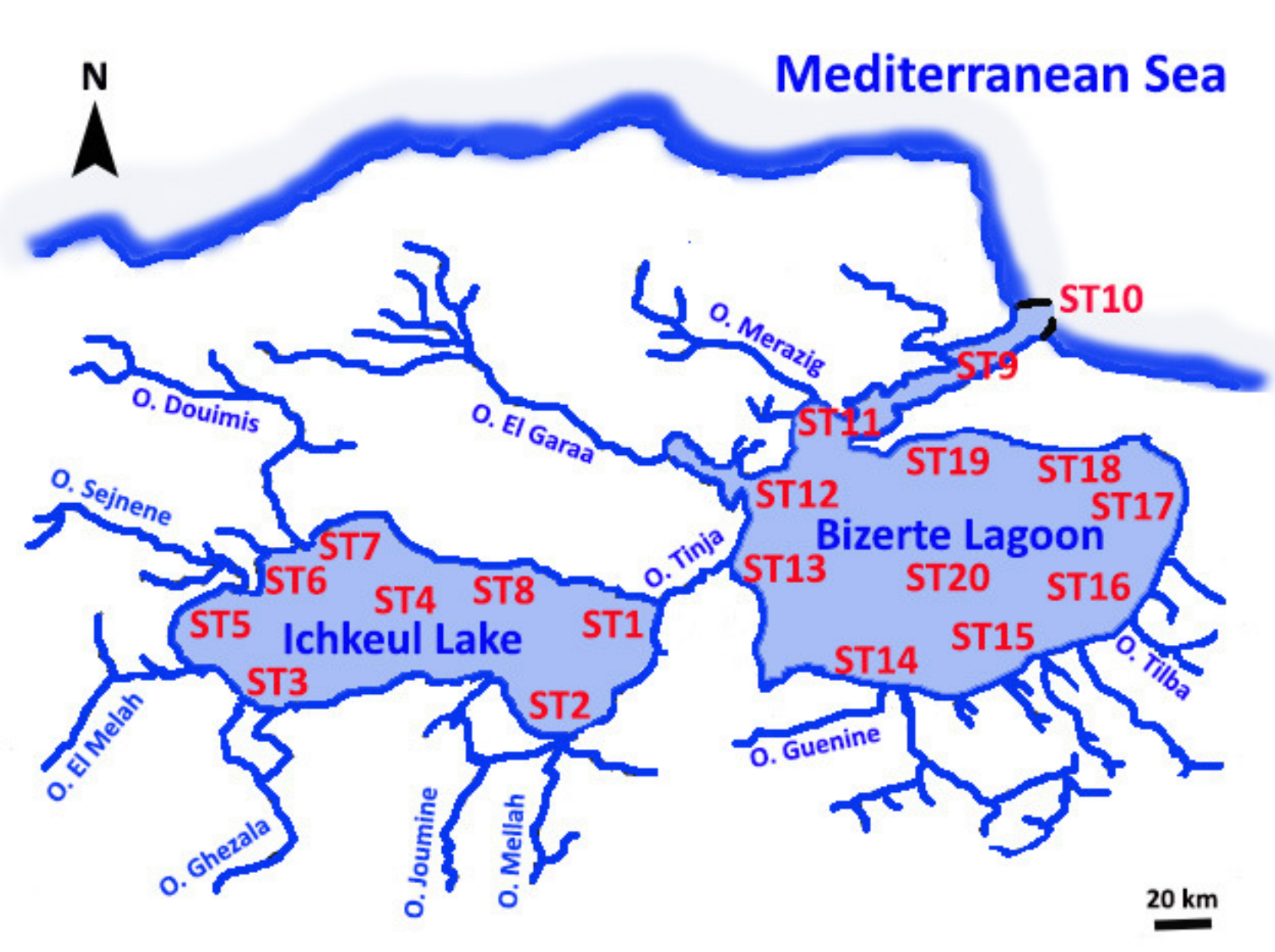
Pollutants (ng g^{-1} dry wt)	Stations			
	ST5	ST8	ST12	ST17
	Lake		Lagoon	
Organic Sn[a]	143±0.3	83±0.3	154±0.1	102±0.5
PCBs[b]	4.66±0.7 (I)[h]	5.58±1 (II)[h]	4.54±0.1 (I)[h]	2.48±0.8 (I)[h]
Heptachlor	10.7±0.7	14±1.6	11±4	12±4
Lindane[d]	268±6	283±13	101±5	95±14
Other OCPs[e]	586±0.03 (IV)[h]	1717±65 (IV)[h]	53±3.4 (III)[h]	86±7.4 (III)[h]
PAHs[f]	1362±560 (II)[h]	444±24 (II)[h]	17988±1183 (IV)[h]	137±26 (I)[h]
m-ERM-Q[g]	8.73±0.03	13.30±0.4	5.30±0.06	3.90±0.1

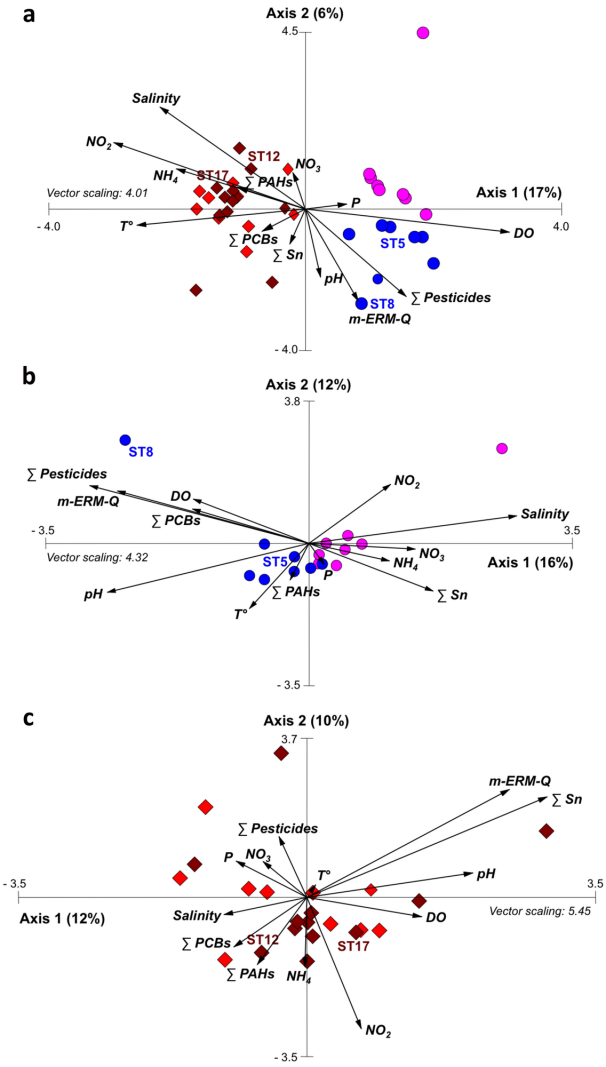
[a]sum of organic forms of tin including methyl, butyl, and phenyl derivatives.
[b]Sum of PCB congeners including PCB18, PCB28, PCB31, PCB44, PCB52, PCB101, PCB138, PCB118, PCB153, PCB149, PCB180, PCB194.
[c]Sum of heptachlor and its epoxide derivative.
[d]Sum of lindane and its metabolites.
[e]Sum of endosulfan, dieldrin, aldrin, chlordane, and their derivatives.
[f]Sum of PAHs including naphthalene, acenaphtylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pryrene, indenol[1,2,3-cd]pyrene, dibenz[a,h]anthracene, and benzoghiperylene.
[g]m-ERM-Q, mean effect range medium quotient calculated according to (Long et al., 1998).
[h]Quality classification according to Bakke et al. (2010).

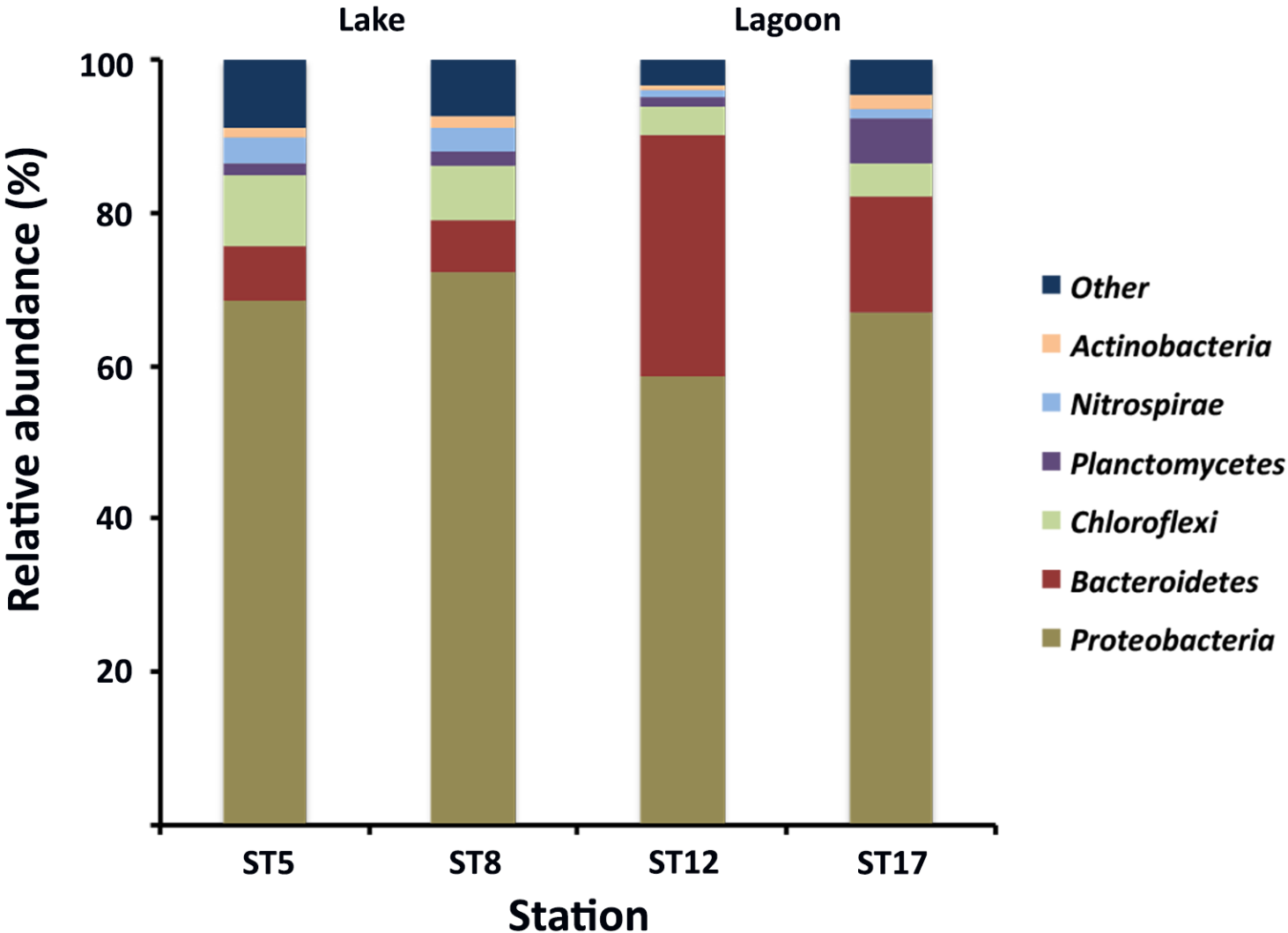
Table 2: Alpha diversity indices (mean ± SD, n=3) of microbial community from the Ichkeul Lake / Bizerte Lagoon stations

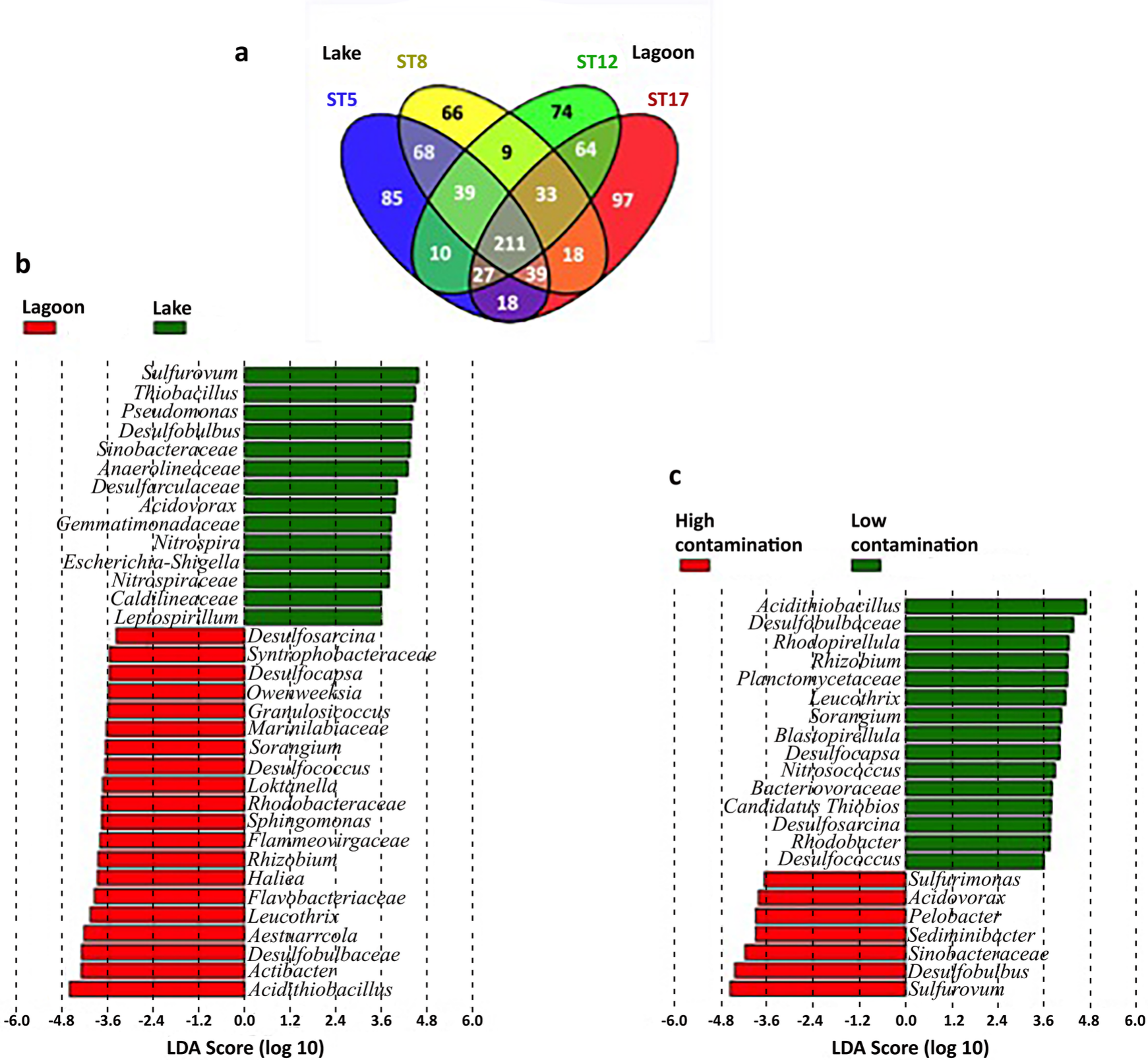
Indices	Station			
	ST5	ST8	ST12	ST17
	Lake		Lagoon	
Reads[a]	22368	19247	22101	25662
Trimmed sequences[b]	16378	14217	15185	18502
Species richness (*R*)	347 ± 32	359 ± 28	341 ± 27	356 ± 40
Coverage	0.93 ± 0.01	0.93 ± 0.01	0.94 ± 0.01	0.94 ± 0.01
Simpson (*1-D*)	0.97 ± 0.01	0.98 ± 0.01	0.96 ± 0.01	0.98 ± 0.01
Shannon (*H*)	4.51 ± 0.1	4.58 ± 0.1	4.37 ± 0.1	4.51 ± 0.05
Evenness (*E*)	0.26 ± 0.04	0.27 ± 0.01	0.23 ± 0.03	0.26 ± 0.02
Chao-1	373 ± 39	386 ± 27	362 ± 27	379 ± 45

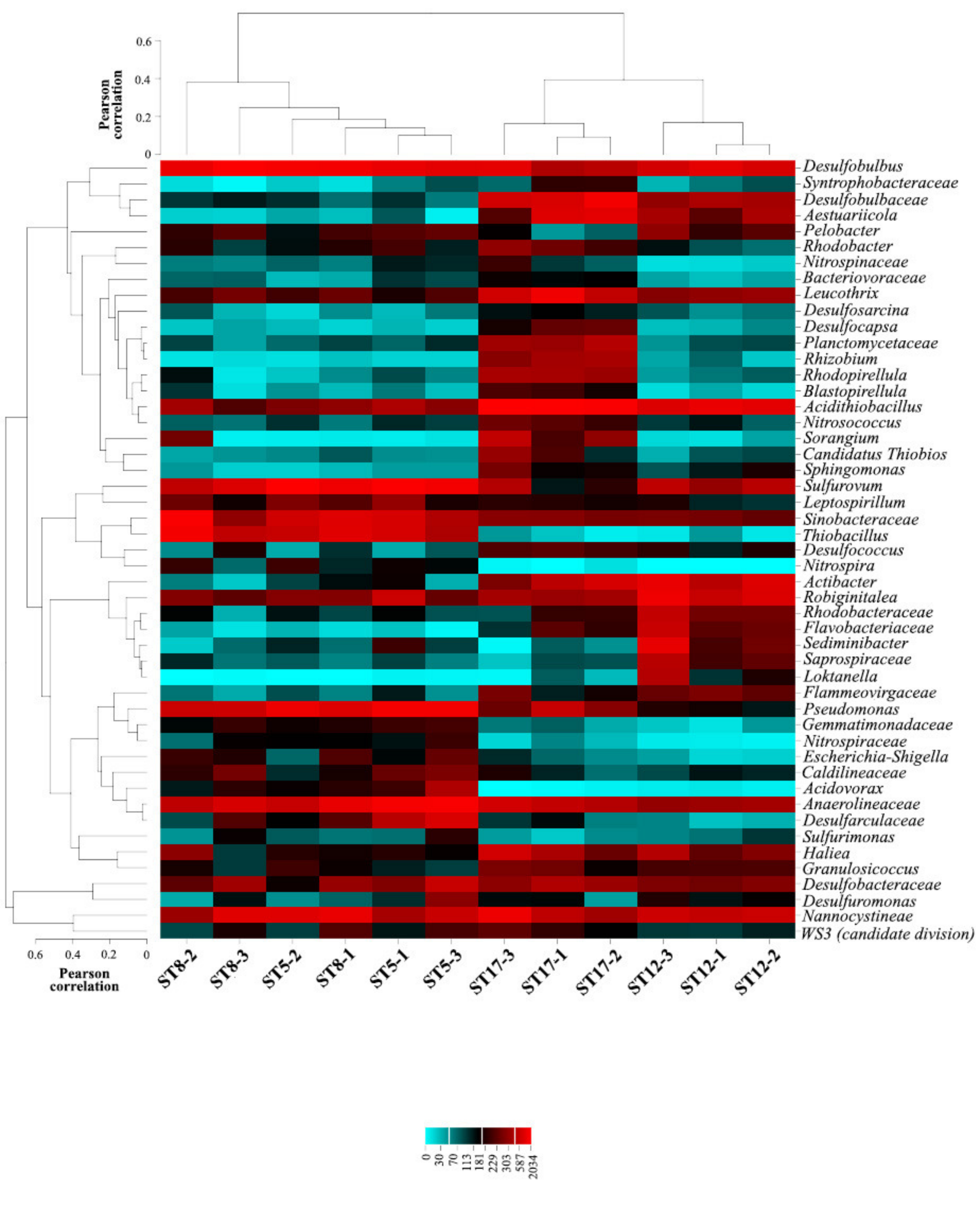
[a]SD: ST5, ± 1966; ST8, ± 1866; ST12, ± 3380; ST17, ± 1688.
[b]SD: ST5, ± 1481; ST8, ± 1406; ST12, ±2708; ST17, ± 1611.

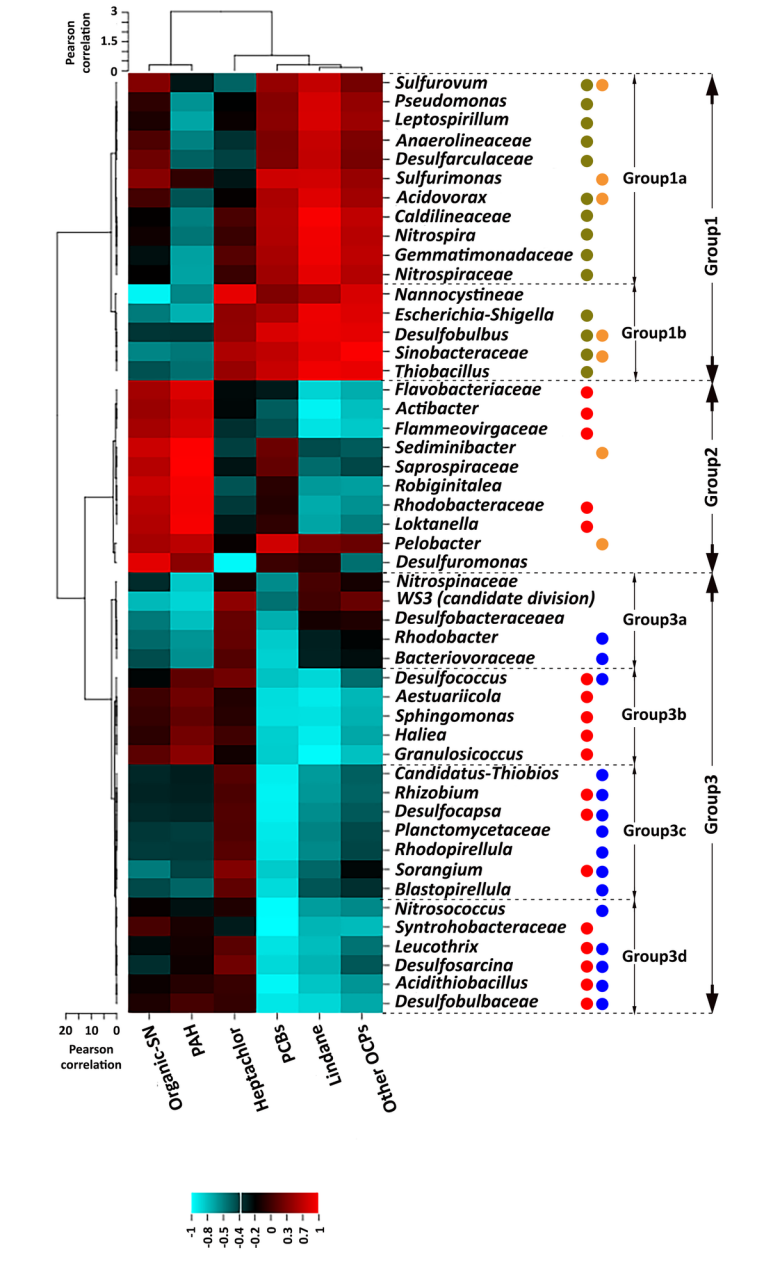

N
Mediterranean Sea
ST10
ST9
O. Merazig
O. Douimis
O. El Garaa
ST11
ST19
ST18
ST12
ST17
Bizerte Lagoon
O. Sejnene
ST7
ST6
ST4
ST8
O. Tinja
ST13
ST20
ST16
ST5
Ichkeul Lake
ST1
ST15
ST3
ST14
O. Tilba
ST2
O. El Melah
O. Guenine
O. Ghezala
O. Joumine
O. Mellah
20 km

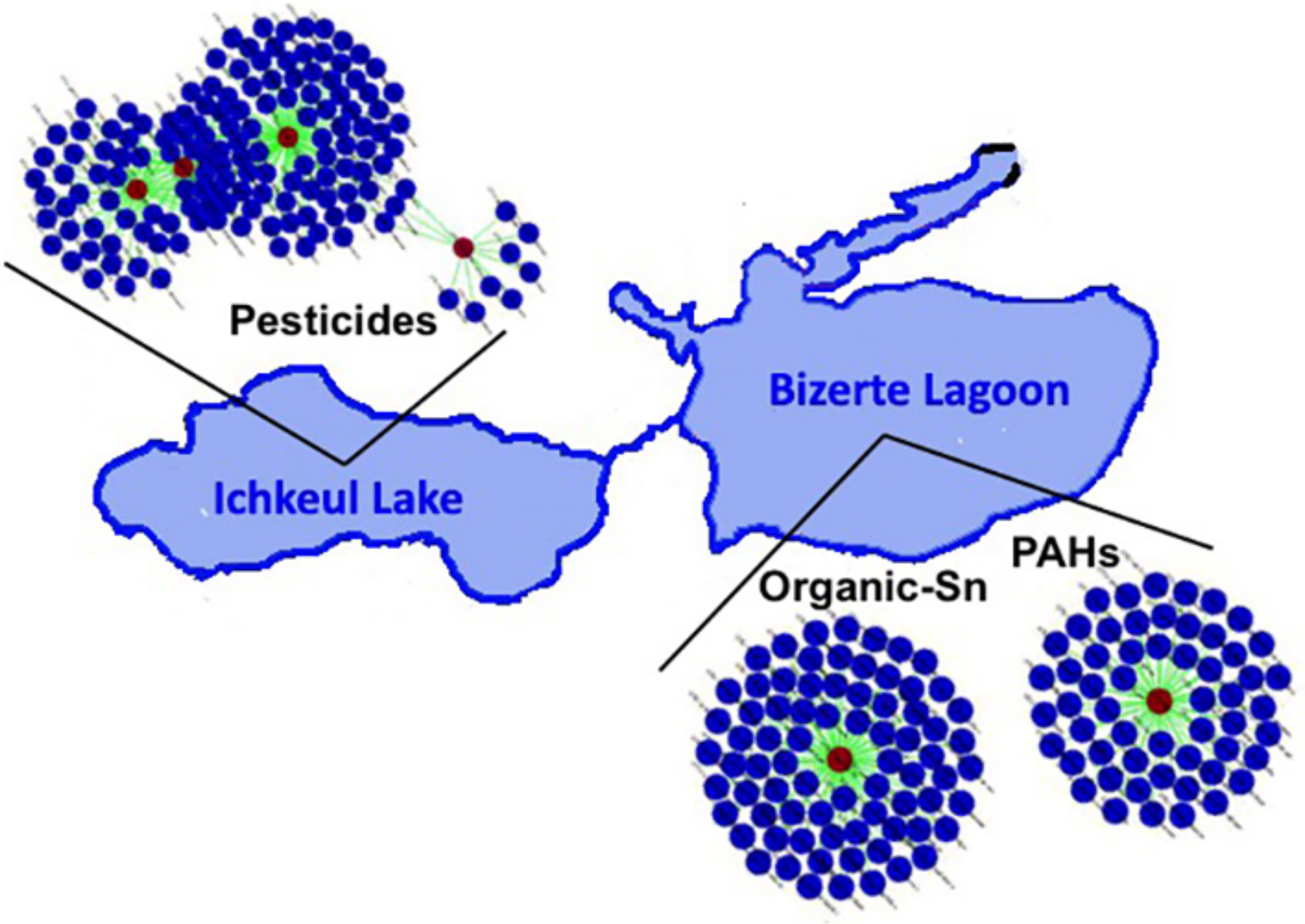

a
Axis 2 (6%)
4.5
- 4.0
Axis 1 (17%)
- 4.0
4.0
Vector scaling: 4.01
Salinity
NO2
NH4
ST17
ST12
NO3
∑ PAHs
P
T°
∑ PCBs
∑ Sn
pH
DO
ST5
ST8
m-ERM-Q
∑ Pesticides
b
Axis 2 (12%)
3.8
- 3.5
Axis 1 (16%)
- 3.5
3.5
Vector scaling: 4.32
ST8
∑ Pesticides
m-ERM-Q
DO
∑ PCBs
NO2
Salinity
ST5
NO3
NH4
P
pH
∑ PAHs
∑ Sn
T°
c
Axis 2 (10%)
3.7
- 3.5
Axis 1 (12%)
- 3.5
3.5
Vector scaling: 5.45
m-ERM-Q
∑ Sn
∑ Pesticides
P
NO3
T°
pH
Salinity
DO
∑ PCBs
ST12
ST17
∑ PAHs
NH4
NO2


Lake
Lagoon
100
80
60
40
20
Relative abundance (%)
ST5
ST8
ST12
ST17
Station
Other
Actinobacteria
Nitrospirae
Planctomycetes
Chloroflexi
Bacteroidetes
Proteobacteria


a
Lake
ST8
ST12
Lagoon
ST5
ST17
66
74
68
9
64
85
39
33
97
211
10
18
27
39
18
b
Lagoon
Lake
Sulfurovum
Thiobacillus
Pseudomonas
Desulfobulbus
Sinobacteraceae
Anaerolineaceae
Desulfarculaceae
Acidovorax
Gemmatimonadaceae
Nitrospira
Escherichia-Shigella
Nitrospiraceae
Caldilineaceae
Leptospirillum
Desulfosarcina
Syntrophobacteraceae
Desulfocapsa
Owenweeksia
Granulosicoccus
Marinilabiaceae
Sorangium
Desulfococcus
Loktanella
Rhodobacteraceae
Sphingomonas
Flammeovirgaceae
Rhizobium
Halioa
Flavobacteriaceae
Leucothrix
Aestuarrcola
Desulfobulbaceae
Actibacter
Acidithiobacillus
-6.0 -4.8 -3.6 -2.4 -1.2 0.0 1.2 2.4 3.6 4.8 6.0
LDA Score (log 10)
c
High contamination
Low contamination
Acidithiobacillus
Desulfobulbaceae
Rhodopirellula
Rhizobium
Planctomycetaceae
Leucothrix
Sorangium
Blastopirellula
Desulfocapsa
Nitrosococcus
Bacteriovoraceae
Candidatus Thiobios
Desulfosarcina
Rhodobacter
Desulfococcus
Sulfurimonas
Acidovorax
Pelobacter
Sediminibacter
Sinobacteraceae
Desulfobulbus
Sulfurovum
-6.0 -4.8 -3.6 -2.4 -1.2 0.0 1.2 2.4 3.6 4.8 6.0
LDA Score (log 10)


Pearson correlation
0.6
0.4
0.2
0
Desulfobulbus
Syntrophobacteraceae
Desulfobulbaceae
Aestuariicola
Pelobacter
Rhodobacter
Nitrospinaceae
Bacteriovoraceae
Leucothrix
Desulfosarcina
Desulfocapsa
Planctomycetaceae
Rhizobium
Rhodopirellula
Blastopirellula
Acidithiobacillus
Nitrosococcus
Sorangium
Candidatus Thiobios
Sphingomonas
Sulfurovum
Leptospirillum
Sinobacteraceae
Thiobacillus
Desulfococcus
Nitrospira
Actibacter
Robiginitalea
Rhodobacteraceae
Flavobacteriaceae
Sediminibacter
Saprospiraceae
Loktanella
Flammeovirgaceae
Pseudomonas
Gemmatimonadaceae
Nitrospiraceae
Escherichia-Shigella
Caldilineaceae
Acidovorax
Anaerolineaceae
Desulfarculaceae
Sulfurimonas
Haliea
Granulosicoccus
Desulfobacteraceae
Desulfuromonas
Nannocystineae
WS3 (candidate division)
0.6
0.4
0.2
0
Pearson correlation
ST8-2
ST8-3
ST5-2
ST8-1
ST5-1
ST5-3
ST17-3
ST17-1
ST17-2
ST12-3
ST12-1
ST12-2
0
30
70
113
181
229
303
587
2034


Pearson correlation
3
1.5
0
Sulfurovum
Pseudomonas
Leptospirillum
Anaerolineaceae
Desulfarculaceae
Sulfurimonas
Acidovorax
Caldilineaceae
Nitrospira
Gemmatimonadaceae
Nitrospiraceae
Nannocystineae
Escherichia-Shigella
Desulfobulbus
Sinobacteraceae
Thiobacillus
Flavobacteriaceae
Actibacter
Flammeovirgaceae
Sediminibacter
Saprospiraceae
Robiginitalea
Rhodobacteraceae
Loktanella
Pelobacter
Desulfuromonas
Nitrospinaceae
WS3 (candidate division)
Desulfobacteraceaea
Rhodobacter
Bacteriovoraceae
Desulfococcus
Aestuariicola
Sphingomonas
Haliea
Granulosicoccus
Candidatus-Thiobios
Rhizobium
Desulfocapsa
Planctomycetaceae
Rhodopirellula
Sorangium
Blastopirellula
Nitrosococcus
Syntrophobacteraceae
Leucothrix
Desulfosarcina
Acidithiobacillus
Desulfobulbaceae
Group1a
Group1b
Group1
Group2
Group3a
Group3b
Group3c
Group3d
Group3
20 10 0
Pearson correlation
Organic-N
PAH
Heptachlor
PCBs
Lindane
Other OCPs
-1 -0.8 -0.5 -0.4 -0.2 0 0.3 0.7 1


Pesticides
Ichkeul Lake
Bizerte Lagoon
Organic-Sn
PAHs