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

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Highlights:

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

Abstract

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

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

INTRODUCTION

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

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

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

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

MATERIALS AND METHODS

Site description

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

Sampling

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

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

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

Physical-chemical analysis and anthropogenic pressure indices

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

Deuterated alkanes (nonadecane $C19d^{40}$ and triacontane $C30d^{62}$ at 10 mg mL^{-1}) and PAH (naphthalene-d^8 and anthrancene-d^{10} at 10 mg mL^{-1}) internal standards were added to each sample and quantification was performed on an Agilent 7890A Gas Chromatography system coupled with a Turbomass Gold Mass Spectrometer with Triple-Axis detector, operating at 70 eV in positive ion mode. External multilevel calibrations were carried out using alkanes [Standard Solution (C8–C40); Sigma], methylated-PAHs (1-methylnapthalene, 2-methylanthracene, and 9,10-dimethylanthracene; Sigma), and PAH (QTM PAH Mix; Sigma) standards, the concentrations of which ranged from 1.125 to 18 mg mL^{-1}. For quality control, a calibration standard (10 mg mL^{-1}) and a blank were analysed every 10 samples. We quantified alkanes between C10 and C36 including pristane and phytane and the following PAHs: naphthalene; all isomers of methyl-, dimethyl- and trimethyl-naphthalenes; acenaphthylene; acenaphthene; fluorene; phenanthrene; all isomers of methyl- and dimethylphenanthrenes/ anthracenes; fluoranthene; pyrene; all isomers of methyl- and

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

For the OCP, quantitative analysis was carried out using selected ion monitoring mode (SIM) as described by Yang et al (2010). The standard of organochlorine pesticide mixture from Supelco consisted of a solution in n-hexane–toluene (1:1, *v*/*v*) at 2,000 μg mL^{-1} of α-hexachlorocyclohexane (α-HCH), β-hexachlorocyclohexane (β-HCH), γ-hexachlorocyclohexane (γ-HCH), δ-hexachlorocyclohexane (δ-HCH), endosulfan α, endosulfan β, endosulfan sulfate, aldrin, dieldrin, endrin, endrin aldehyde, endrin ketone, heptachlor, heptachlor epoxide, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane, (4,4_-DDT), 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane (4,4_-DDD), 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (4,4_-DDE), methoxychlor, and hexachlorobenzene (HCB). A standard solution of 2,4,5,6-tetrachloro*m*-xylene and dibutylchlorendate in n-hexane–toluene (1:1, *v*/*v*) at 2,000 mg mL^{-1} was purchased from Supelco to use as internal standard. The concentration range of each pesticide was from 0.15 to 2 μg L^{-1} and the internal standards used in quantification at a concentration of 1 μg L^{-1}.

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

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

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

advantage to be integrative. It is based on the average of normalized threshold effects levels (TEL) for each individual pollutant considered (OCPs, PAHs, alkanes, and metals) effective to estimate the toxic effect of pollutants in aquatic ecosystem (Aylagas et al., 2017).
The threshold values used for determining the Pi were based on the TEL from the National Oceanic and Atmospheric Administration (NOAA 1999) presented in supplementary Table S2. Pi values range between 0 and 5 where 0 corresponds to a total absence of pollutant pressure and 5 corresponds to the maximum pressure. The ecological status was evaluated using the microbial genomic AZTI's Marine Biotic Index (microgAMBI index), calculated as previously reported (Aylagas et al., 2017; Borja 2018).

DNA extraction, PCR amplification and Sequencing

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

Sequence data analysis

Read-pair overlapping, sequence data filtering and clustering into SWARM Operational Taxonomical Units (OTUs), was done for each sample as previously described by Lanzén et al. (2020). Briefly, reads were overlapped using *vsearch* v2.7.1 (Rognes et al., 2016). Primers were then removed using *cutadapt* v1.15 (Martin 2011). Remaining sequences were de-replicated and sorted by abundance using *vsearch.* SWARM v2.2.1 (Mahé et al., 2015) was then used to

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

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

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

Statistical analysis

Statistical analyses were performed using R packages vegan (Oksanen et al., 2013). Alpha diversity indices of microbial community were calculated based on the rarefied OTU table. The

differences between these indices were tested by analysis of variance (ANOVA). Differences among group means were tested using least significant difference test LSD, function LSD.test (used for multiple comparisons) within "agricolae" package after ANOVA test. Permanova (permutational multivariate analysis of variance) was performed to estimate the effect of factors and their interactions on the microbial communities (999 permutations). ANOSIM (analysis of similarities) was performed to compare samples. SIMPER analyses based on Bray-Curtis similarity matrix were carried out to determine the OTUs contribution and their differences between sites, stations and seasons. Venn diagram was visualized with InteractiVenn (Heberle et al., 2015), based on the distribution of OTUs showing specific and shared OTUs between sites, stations and seasons.

Community similarity was assessed by Non-Metric multidimensional Scaling (NMDS) correlating prokaryotic communities with physical-chemical parameters and chemical compounds. The sum of the different type of contaminants (∑ alkanes, ∑ PAHs, ∑ Metals and ∑ OCPs), each individual metal, and Pi were used in order to consider the multi-contamination for each type contaminant. The significant chemical variables were fitted to the NMDS as vectors with the envfit function on R Statistics software.

Linear discriminant analysis effect size (LEfSe) (Segata et al., 2011) was applied to determine seasons and sites specific biomarker. It performs several tests to assess their reliability. Firstly, a Kruskal-Wallis (KW) sum rank non-parametric test was performed to detect significant differential taxa abundance (p <0.05). A pairwise Wilcoxon test was performed to investigate the biological consistency (p <0.05). The linear discriminant analysis (LDA) logarithmic threshold was setup to 2. A heatmap-clustering correlation was performed with CIMminer (http://discover.nci.nih.gov/cimminer/). These analyses were performed at the genus level to determine the relation between pollutants concentrations and taxon abundances and genus rank.

RESULTS AND DISCUSSION

Contamination level

The principal component analysis (PCA), based on physical-chemical parameters (granulometry, ions, C and N contents…), indicated that the compartments (soil, river, and lake) shared similar characteristics (supplementary material, Fig. S2 and Table S1), suggesting

limited influence of physical-chemical parameters in shaping the microbial community structure. Such observation indicated a soil-river-lake continuum with exchange of material probably via winter rain runoffs as the Ichkeul Lake is a receptacle of the surrounding watersheds (Ben Salem et al., 2016a). Accordingly, the PCA also distinguished lake in summer by high content of chloride and sodium ions, while most soil in winter were rich in carbonate (supplementary material, Fig. S2 and Table S1). Such result was not surprising since the Ichkeul Lake receives runoff inputs from winter rains while the water level decreases in summer increasing salinity in summer (dry season). From the physical-chemical analyses (supplementary material, Table S1) for samples collected in September 2018 (summer, dry season) and in April 2019 (winter, wet season) at the three stations (S2, S3, and S6) the sums of the different types of pollutants were calculated (Table 1). All the stations contained higher metal concentrations than PAHs, alkanes and OCPs. The metal concentrations ranged between 300 ± 55 to 2421 ± 2457 mg kg^{-1} in summer and 217 ± 20 to 2536 ± 677 mg kg^{-1} in winter. Metal concentrations were significantly different between compartments (two-way ANOVA, p <0.001), but not between seasons (p > 0.05). In contrast, the organic contaminants (PAHs, Alkanes and OCPs) showed different concentrations according to compartments (two-way ANOVA, p <0.001) and seasons (two-way ANOVA, p < 0.01). Total PAHs concentrations ranged from 288 ± 4 to 1210 ± 20 ng g^{-1} and from 322 ± 4 to 1436 ± 26 ng g^{-1} in summer and winter respectively, being higher in winter than in summer (two-way ANOVA, p <0.001), while the alkane concentrations were higher (two-way ANOVA, p < 0.001) in summer (from 193 ± 8 to 637 ± 10 ng g^{-1}) than in winter (from 301 ± 27 to 1127 ± 63 ng g^{-1}). The OCP concentrations showed similar trend as alkane concentrations, being higher in summer than in winter (two-way ANOVA, p < 0.01). The concentrations of organic contaminants (PAHs, Alkanes and OCPs) were higher in soil than in river and lake sediments (two-way ANOVA, p <0.001), while the metal concentrations were higher in river and lake sediments than in soil (two-way ANOVA, p <0.01). Also, the contaminant concentrations were different between river and lake sediments (two-way ANOVA, p <0.001). Such observations correlated with the on-going anthropogenic activities taking place around the Ichkeul Lake, such as the presence of an oil refinery near S2 (Ben Said et al., 2010), intensive agriculture practices near S3 (Stevenson and Battarbee 1991) and a wastewater discharge from an iron mine plant in an upstream location (Touaylia et al., 2016). Accordingly, the ratio FLA/(FLA+PYR) was found to be between 0.4 ± 0.01 and 0.8 ± 0.1 indicating the

pyrogenic origin of PAHs by combustion of both petroleum and biomass, as previously described (De La Torre-Roche et al., 2009). Although the diagnostic ratio should be considered with caution, the FLA/(FLA+PYR) ratio, suitable for the identification of PAH sources in soil and sediments (Tu et al., 2018), further demonstrated the human impact on the Ichkeul Lake area. In accordance with the PAH concentrations trend, the FLA/(FLA+PYR) ratio showed differences according to compartments (two-way ANOVA, p <0.001) but not to seasons (two-way ANOVA, p > 0.1). In order to estimate the toxic effect of pollutants on the ecosystem, the Pressure index (PI), integrating the toxic effect of all individual pollutants (Aylagas et al., 2017), was calculated. The Pi values of the compartments (soil, river and lake from each station) also indicated a moderate pressure (Table 1). The Pi values were different according to compartments (two-way ANOVA, p <0.01) and seasons (two-way ANOVA, p <0.05). The soil at station S3 in winter was the most contaminated (Pi= 2.93 ± 0.15), while the sediment river at station S6 in summer was the less (Pi= 1.46 ± 0.2). Although the contamination level was slightly divergent in all sites, it is interesting to note that all sites showed a similar trend based on the Pi values as follows: sediment river > sediment lake > soil (Table 1).

Prokaryotic community structure and composition

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

All stations, irrespective of the compartments, were dominated by Proteobacteria, Actinobacteria, Bacterioidetes, and Chloroflexi (Fig. 2). These phyla have been detected as dominant in Ichkeul Lake/Bizerte Lagoon hydrological system (Ben Salem et al., 2019) and in diverse environments, including river sediments (Sun et al., 2013) and soils (Lin et al., 2019). They have been described playing a key role in biogeochemichal cycles (Andrews et al., 2011) and in contaminant degradation, including petroleum hydrocarbons (Guermouche M'rassi et al., 2015) and pesticides (Magnoli et al., 2020). The microbial community composition of the soil samples appeared similar regardless of the season (ANOSIM, p > 0.05) but differed according to the stations (ANOSIM, p < 0.01). In contrast, the composition

of the river and the lake sediments shifted between summer and winter (ANOSIM, $p < 0.05$). Noteworthy, the lake sediments showed specific patterns in summer (dry season) that were different (ANOSIM, $p < 0.01$) to those observed in winter (wet season, Fig. 2). Nonetheless, microgAMBI showed similar ecological status, ranging from good to moderate, as shown by the Pi (Table 1 and 2), revealing that bacterial communities responded to the pollution levels as detected by Aylagas et al. (2017). The comparison of alpha diversity indices did not reveal significant differences (Two-way ANOVA, $p > 0.05$) between the samples (Table 2). The adaptation of the microbial communities to pollutants (legacy effect) and their mixing (coalescence) may explain the fact that microbial diversity was similar in all samples, in line with previously reported results (Jurburg et al., 2017; Nunes et al., 2018; Yin et al., 2015).

The comparison of the microbial communities by NMDS analysis separated most of the soil, river sediment and lake sediment into distinct compartment-specific clusters (Fig. 3A), highlighting their different composition. Such observation was further supported by Permanova showing that the microbial diversity variation was explained mainly by the compartment origin ($p < 0.002$; Fig 3B), the bacterial communities from soil being significantly different from that of sediments (Permanova, $p < 0.002$). The bacterial communities of river sediment showed significant difference with that of lake sediment (Permanova, $p < 0.01$). However, the microbial communities of river sediments from S2 in summer was clustered far from other river sediment samples, close to lake sediments. This may be explained by the fact that the sampling sites in river and lake were closer at site S2 (Fig. 1). Nevertheless, the soil cluster was closer (Fig. 3A; ANOSIM, $p < 0.001$) to the river sediment cluster (SIMPER: 26 %) than that of the lake sediment (SIMPER: 5 %), consistent with the soil-river-lake continuum. When comparing communities between the two seasons, differences were observed within the environmental compartments (Permanova, $p < 0.01$), the lake cluster being the more dispersed, particularly for stations S2 and S3 (Fig. 3). This confirms the seasonal variations of communities observed in the Ichkeul Lake sediments (Ben Salem et al., 2019). It is also consistent with the physical-chemical variations observed in the Ichkeul Lake (supplementary material, Table S1), showing salinity modification according to the hydrology of the Ichkeul Lake receiving saline water from Bizerte Lagoon in summer (Ben Salem et al., 2017). It has been demonstrated that salinity is a main driver for community organization (Xi et al., 2014), with its fluctuation drastically affecting microbial community composition and structure (Ben

Salem et al., 2019). The NMDS analysis substantiated the capacity of Pi and microgAMBI to reveal an agreement between the ecological status (good and moderate) and the level of pressure (low-moderate; Tables 1 and 2), indicating that lake sediment was the more exposed to contaminants than river sediment and soil (Fig. 3). Consistently, the NMDS showed that the Total PAHs (∑ PAH) influenced the microbial communities of the lake sediment. This agrees with previous reports showing that this contaminant affects microbial communities in diverse environments (Giloteaux et al., 2010, 2013; Vercraene-Eairmal et al., 2010), especially in coastal areas (Duran et al., 2015a,b; Pringault et al., 2008). Consistently, the Venn diagram (supplementary material Fig. S3) showed an average of 41.3 ± 3.73 shared OTUs between compartments (soil, river and lake) in each station (S2, S3 and S6) considering both seasons. These shared OTUs correspond to the core community at the stations, further confirming the connectivity along the soil-river-lake continuum. These observations were supported by SIMPER analyses, especially for the core community composition (SIMPER: 10 %). The Venn diagram also showed a core community for each season (supplementary material Fig. S3), more important in winter 24 ± 22 OTUs (SIMPER: 34 %) than in summer, 1.3 ± 1.2 OTUs (SIMPER: 34 %). The seasonal comparison of the communities in the compartments (soil, river and lake) revealed seasonal stable OTUs (shared OTUs by a compartment irrespective of the season), which number was higher in soil 960 ± 145 OTUs (SIMPER: 47 %), than in both aquatic ecosystems, lake 716 ± 533 OTUs (SIMPER: 44 %) and river 471 ± 388 OTUs (SIMPER: 42 %) indicating that soil represents a habitat where communities are better established. This latter observation is in accordance with the fact that, as receptacle compartments, aquatic environments are more susceptible to variations as further indicated by a high number of specific OTUs in the lake (3182 ± 682 OTUs) and river (1517 ± 467 OTUs) than in soil (1306 ± 232 OTUs).

Linear discriminant analysis effect size (LEfSe) revealed a difference in taxa abundance according to the compartments (soils, rivers and lakes) and seasons. LEfSe exhibited specific genera for each habitat that were more abundant ($p < 0.05$) in soils than in both aquatic ecosystems (Fig. 4A). The habitat specificity was consistent with previous studies for microbial communities in coastal areas (Ben Salem et al., 2019; Jeanbille et al., 2016a; Xi et al., 2014) and soils (Chaudhary et al., 2015; Dong et al., 2019). Moreover, LEfSe showed a specificity of genera in each season/compartment that were more frequent ($p < 0.05$) in lakes habitats (Fig.

4B). The presence of particular genera, such as *Arenimonas*, *Bryobacter* and *Salinimicrobium,* that were found dominant ($p < 0.05$) in lake sediments in winter, were also revealed dominant ($p < 0.05$) in soil (*Arenimonas* in winter, and *Bryobacter* in summer) and in river (*Salinimicrobium* in summer) by LEfSe (Fig. 4B). Such observation also suggested the transfer of microorganisms from a compartment to another along the soil-river-lake continuum. Interestingly, several members of the *Arenimonas* genus have been isolated from ecosystems located at the soil/aquatic ecosystems interfaces, including sandy beaches (Kwon et al., 2007), estuaries (Jeong et al., 2016), and rice-fields (Zhang et al., 2015, 2017). Similarly, some members of the halophilic genus *Salinimicrobium* have been isolated from coastal sediments (Lee et al., 2012; Zhang et al., 2017), and from saline and reclaimed soils (Chen et al., 2008; Kim et al., 2016), while *Bryobacter* genus has been described mainly in soils and paddy soils (Kulichevskaya et al., 2010; Li et al., 2021). Thus, our study provides compelling evidence on the ecophysiology and ability of these taxa in colonizing and thriving within the mixing zones of the soil-river-lake continuums according to the season.

Impact of pollutant on microbial communities

The cluster correlation (ClusCor) analysis revealed the correlations between the abundance of individual prokaryotic genera and the concentrations of environmental pollutants (Fig. 5). The analysis revealed four main groups. The Group G1, showing strong positive correlations ($0.5 <$ Pearson correlation < 0.8, $p < 0.05$) with Hg, As, Zn, Pb and the sum of metal concentrations, included *Nitrospira*, *Arenimonas*, *Dinghuibacter*, *Altererythrobacter*, *Microbacterium*, *Flavisolibacter*, and *Agromyces* genera (Fig. 5). Interestingly, the LEfSe analysis showed that these genera were found significantly more abundant ($p < 0.05$) according to the compartment and/or the season (Fig. 4) suggesting that they play a crucial role as specialists in metal contaminated environments. Consistently, members related to these genera have been found predominant in metal contaminated environments, as for example *Microbacterium, Agromyces*, and *Dinghuibacter* in soils (Corretto et al., 2017, 2020; Lv et al., 2016; Xiao et al., 2019), *Nitrospira* and *Flavisolibacter* in soil and freshwater ecosystems (Das et al., 2016; Hong et al., 2015; Liu et al., 2018), and *Arenimonas* and *Altererythrobacter* in marine sediments (Jeong et al., 2013; Matsumoto et al., 2011). Thus, our results show that

various taxa able to cope with metals are present in the different habitats along the soil-river-lake continuums, and there are most of the time metal specific

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

The Group G3 gathered genera which abundance correlated with the presence of organic compounds (Fig. 5). Considering the strongest positive correlation (Pearson correlation > 0.6, $p < 0.05$), three subgroups were revealed according to the nature of the main correlated compound. The "pesticide subgroup", correlated with OCPs, included *Rubrobacter*, *Gaiella*, *Gemmata*, and *Microvirga* genera, which were shown significantly more abundant ($p < 0.05$) in the soil compartment by LEfSe (Fig. 4A), together with *Skermanella* and *Blastococcus* genera (Fig. 5). Such observation was consistent with previous reports indicating that members of these genera were found to be predominant in pesticide treated soils (Kumar et al., 2020; Liu et al., 2020; Thelusmond et al., 2016), where they play important functional roles related to the nitrogen biogeochemical cycle (Aguiar et al., 2020; Yang et al., 2019). These genera were also correlated (Pearson correlation > 0.5, $p < 0.05$) with Cd and Cu (Fig. 5), metals introduced in agricultural soils by the use of Cd-rich phosphate fertilizers (Li et al., 2020b) and copper antimicrobial compounds (Lamichhane et al., 2018). They have also been described in metal-contaminated soils (Liu et al., 2019a; Liu et al., 2019b; Liu et al., 2021). It is likely that the genera of the G3 “pesticide subgroup” own metabolic properties well adapted to the presence

of agricultural contaminants such as OCPs and metals. *Massilia* genera and genera related to *Blastocatellaceae* RB41, that were shown significantly more abundant in the soil compartment by LEfSe ($p < 0.05$), correlated (Pearson correlation > 0.8, $p < 0.01$) with PAHs constituting the "PAH subgroup" (Fig. 5). Members of these genera are known for their capacities to degrade complex organic compounds (Huber et al., 2014; Pascual et al., 2015), including PAHs (Araujo et al., 2020; Bodour et al., 2003; Duran et al., 2015a). Finally, the *Acidibacter* genus formed the "alkane subgroup", also significantly found to be more abundant ($p < 0.05$) in the soil compartment (LEfSe, Fig. 4A), consistently with the fact that members of this genus have been detected in oil-contaminated soils (He et al., 2020). Noteworthy, the genera constituting the three subgroups of Group G3 were found to be predominant in the soil compartment (LEfSe, $p < 0.05$, Fig. 4A) suggesting that these genera might play a pivotal role to cope with the presence of organic compounds in soils. Beside these three subgroups, Group G3 included genera showing significant positive correlation (Pearson correlation > 0.5, $p < 0.05$) with "alkanes" (*Pirellula*) and "alkanes + PAHs" (*Opitutus*, *Fusibacter*, and *Lysobacter*) or "OCPs" (*Nocardioides*, *Gemmatimonas*, and *Bryobacter*) and "OCPs + PAHs" (*Adhaeribacter* and *Solirubrobacter*) (Fig. 5). Interestingly, LEfSe showed that the genera correlated with petroleum hydrocarbons alone ("alkanes" and "alkanes + PAHs") were predominant in river sediment ($p < 0.05$) while most genera correlated with pesticides and petroleum hydrocarbons ("OCPs" and "OCPs + PAHs") were predominant in soil ($p < 0.05$, Fig. 4). Such observation was in accordance with the contamination levels in the different compartments (Table 1). It is likely that these "specialist" bacteria, able to cope with the presence of contaminant type, are habitat (soil, river, and lake compartments) dependent. Such habitat specificity, described in previous studies (Ben Salem et al., 2017; Guo et al., 2016; Jeanbille et al., 2016a,b; Smii et al., 2015), is highlited in our study. It was particularly noticeable for *Pirellula*, *Fusibacter*, *Nocardioides,* and *Lysobacter* genera for river sediment, while *Adhaeribacter* and *Solirubrobacter* were specifically linked with soil (LEfSe, $p < 0.05$, Fig. 4). These genera have been proposed as pollution indicators: *Pirellula*, *Fusibacter*, and *Nocardioides* genera for freshwater sediments (Chen et al., 2016; Guo et al., 2016), while *Lysobacter, Adhaeribacter* and *Solirubrobacter* genera for chlorinated pesticide polluted soils (Aguiar et al., 2020; Cheng et al., 2020; Ramírez et al., 2020). Thus, despite the connection between compartments, our results indicated that these genera remained habitat specific. In contrast, *Gemmatimonas* and *Bryobacter* genera correlated with the presence of OCPs irrespective of the habitat, as they

were found predominant in soil and lake sediment compartments (Fig. 4). These genera are described cosmopolitan being found in diverse aquatic and terrestrial ecosystems (Li et al., 2021; Zeng et al., 2016). The presence of these genera, able to inhabit various ecosystems, in the different compartments supports the connection between compartments within the soil-river-lake continuum, suggesting that they can be transferred from a compartment to another via running waters and runoff (Luo et al., 2020), which are important in winter in the Ichkeul Lake area (Ben Salem et al., 2016a).

Among genera included in the Group G4, three main subgroups were noticeable considering the strongest correlations: i) *Actibacter*, *Anaerolinea* and *Thiobacillus* genera showing significant positive correlations (Pearson correlation > 0.7, $p < 0.01$) with mercury content. *Actibacter* was found significantly abundant in aquatic sediments, *Thiobacillus* in the river and soil compartments (LEfSe, $p < 0.05$), while LEfSe revealed no specific habitat for *Anaerolinea* (Fig. 4). Interestingly, *Anaerolinea* has been detected in mercury contaminated soil/water transition zone (Du et al., 2020), being more abundant in sediment than in soil. It is likely that this genus is well adapted to the variation of environmental parameters as that occurring in mixing zone such as soil-river-lake continuum. The metabolic capacities allowing *Anaerolinea* to occupy diverse habitats with the presence of mercury deserve to be further investigated to elucidate its ecological role. Among these three genera only *Thiobacillus* has been described able to transform mercury species (Huang et al., 2020; Vázquez-Rodríguez et al., 2015). *Thiobacillus* carry *mer* genes operon on either chromosome or plasmid (Velasco et al., 1999), the later allowing the spreading of *mer* genes within the microbial community by horizontal gene transfer (Mindlin et al., 2002). Horizontal gene transfer has been recognized as important adaptation mechanism providing “immunization” against pollutants to microbial community (Poursat et al., 2019), which advocate functional gene analyses for the development of microbial bioindicators of pollution. Noteworthy, the abundance of these genera showed negative correlation (Pearson correlation < -0.5, $p < 0.05$) with organic compounds (OCPs and PAHs) content, consistent with the fact that *Anaerolinea* has been described sensitive to the presence of high molecular weight PAHs (Júlio et al., 2019). Such exclusion to organic compounds further confirmed these genera as mercury “specialists”.

ii) *Pontibacter*, *Nafulsella, Salinimicrobium*, *Cesiribacter* showing significant positive correlation (Pearson correlation > 0.5, $p < 0.05$) with Ni and Cr, and significant negative

correlation (Pearson correlation < -0.05, p < 0.05) with PAHs (Fig. 5). Such observation suggested that these four genera are probably "specialists" well adapted to the presence of Ni and Cr as they excluded with other pollutants. The LEfSe analysis (Fig. 4) revealed no specific habitat for the *Nafulsella* genera, while *Pontibacter*, *Salinimicrobium*, *Cesiribacter* were found to be dominant in the aquatic compartments (lake and/or river, p < 0.05). Although information on these genera is scarce regarding metallic contaminants, the fact that *Pontibacter* has been detected in Cr-polluted soils (García-Gonzalo et al., 2017) reinforce our observation. iii) the *Bacillus* genus showing strong positive correlation (Pearson correlation > 0.5, p < 0.05) with PAHs, revealed predominant in lake sediment by LEfSe (p < 0.05, Fig. 4A). It is likely that this genus has an important role in the Ichkeul Lake/Bizerte Lagoon complex as it has been detected in several sampling campaigns (Ben Said et al., 2008; Louati et al., 2013).

The ClusCor analysis showed four main groups of genera (G1-4) showing either co-occurrence or exclusion with pollutant in the different compartments, which represent potential bio-indicators for assessing ecosystem health as previously proposed (Lladó et al., 2018; Savvaidis et al., 2001; Segata et al., 2011). This information is useful for establishing ecological status of an ecosystem by including the bio-indicator genera in databases used for calculating microbial indices such as microgAMBI (Aylagas et al., 2017; Borja 2018). The observed links between the abundance of specific genera and contaminants suggested that identified genera might have metabolic capacities conferring a specific role in pollutant transformation or degradation and ecosystem functioning that, in turn, influence the overall microbial community assemblage, which also depends on environmental parameters such as pH, nutrients and oxygen availability. In order to unveil the specific functional role of the four groups of genera, their functional capabilities were inferred by PRICRUSt2 analysis focusing on specific functions related to the presence of pollutants, including hydrocarbon and pesticide degradation, and metal transformation or resistance. The nearest sequenced taxon indices (NSTI) ranged between 0.09 and 0.29 indicating the reliability of the PICRUSt2 prediction analysis. The abundance distribution (supplementary material, Fig. S4) and the comparison of the identified genera groups, based on their predicted functional capabilities, revealed that they shared most of the functions related to pollutants (Fig. 6). Consistently with the ClusCor analysis (Fig. 5) the groups G1 and G3 were specifically linked with genes involved in metal transformation/resistance, as for example: K19591 and K19592 (Hg and Cu resistance),

K08364 and K08365 (Hg resistance), and K015726 and K015727 (Co, Zn and Cd resistance) for Group G1, while Group G3 was linked with As (K11811), Zn (K13638), and Hg (K08363 and K00520) resistance genes (supplementary material, Table S3). The differences on metal transformation/resistance genes between Group G1 and Group G3 probably reflect the habitat specificity: members of the Group G1 being more cosmopolitan (found in the three compartments) than those of Group G3, which were particularly found to be more abundant in the soil compartment (Fig. 4).

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

Interestingly, the groups G1 and G2 were linked with genes connected with the degradation of aromatic compounds. The Group G1 being associated with di- and mono-oxygenase (K04100, K04101, and K03380) genes, whilst the Group G2 was linked with genes involved in PAH degradation including dehydrogenase (K13954), hydroxylase (K00480), decarboxylase (K04102), and CytP450 (K14338) genes, which was consistent with the ClusCor analysis showing the correlation of Group G2 members with PAH content (Fig. 5). As observed for metals, the differences shown by Group G1 and Group G2 regarding PAH degradation genes might reflect the habitat specificity as members of the Group G2 were specifically found to be more abundant in the aquatic compartments (Fig. 4).

The Group G4 was linked with genes contributing to anaerobic metabolism such sulfate reduction (K11181) and nitrogen fixation (K02586, K02588, and K02591), together with genes participating to the aerobic degradation of aromatic compounds (supplementary material, Table S3), including CytP450 (K01253), dehydrogenase (K10217), and dioxygenase (K05784) genes. The fact that Group G4 encompassed anaerobic and aerobic metabolisms was

consistent with the LefSe analysis showing that Group G4 included genera with specific habitat (soil, river or lake compartment) together with genera without habitat specificity (Fig. 4).

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

In this study, the comparison of microbial community composition along the different transects of the soil-river-lake continuums revealed a relatively large number of shared OTUs. Such observation indicated bacterial community coalescence where the soil microbes, and the suspended matter entering the aquatic ecosystem through runoff are mixed with the microbial population inhabiting river and lake sediments. Moreover, the study revealed that the organisation of microbial communities was shaped by the presence of pollutant and by habitat filtering. Microbial genera groups were identified as "specialist" taxa exhibiting key degradation/transformation functions involved in metabolisms activated in response to the presence of pollutants. The study provides novel information on microbial community organisation in soil-river-lake continuums submitted to multi-contamination. The results obtained in this study pave the way for future investigations to better understand the organisation and functioning of microbial communities in soil-river-lake continuum mixing zone, where microorganisms and organic materials are transferred from a compartment to another. The expected information will be of paramount importance for the development of bio-indicators reporting on the recovery capacities of a contaminated ecosystem. Several studies propose microbial biomarker of contamination (Ben Salem et al., 2019; Ford et al., 2005; Ventorino et al., 2018), particularly integrative microbial indices such as microgAmbi

(Aylagas et al., 2017; Borja 2018) that in our study is consistent with the chemical pressure index. Including specialist microorganisms and considering also their metabolic capacities in such integrative indices would help to develop accurate biomarker of contamination (Ventorino et al., 2018). However, several environmental factors (e.g. pH, nutrients and oxygen availability) affect the microbial community composition and structure (Duran et al. 2015a,b), which have to be considered when developing biomarkers.

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Declaration of competing interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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Figure caption

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

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

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

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

Fig. 5. Correlation between microbial OTUs and pollutant concentrations by clustered correlation (ClusCor) analysis. 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. 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 according to compartment: soil (blue circle), river (green triangle) and lake (red square); or the season: summer (full symbol) and winter (empty symbol). Σ Alkanes, sum of

alkane compounds; Σ PAHs, sum of polyaromatic hydrocarbons; Σ OCPs, sum of organochlorine pesticides; Σ metals, sum of metals; Pi, pressure index.

Fig. 6. Comparison of PICRUSt-predicted pollutant degradation/transformation capabilities of the genera groups (G1-G4). The genera groups (G1-G4) identified by the ClusCor analysis (Fig. 5) were compared by NMDS based on PICRUSt-predicted genes involved in pollutant degradation/transformation, including metals (red), pesticides (green), and hydrocarbons (black). The most relevant genes specifically abundant in each genera group discussed in the text are identified by numbers and their description provided in supplementary material Table S3.

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

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

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

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

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

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

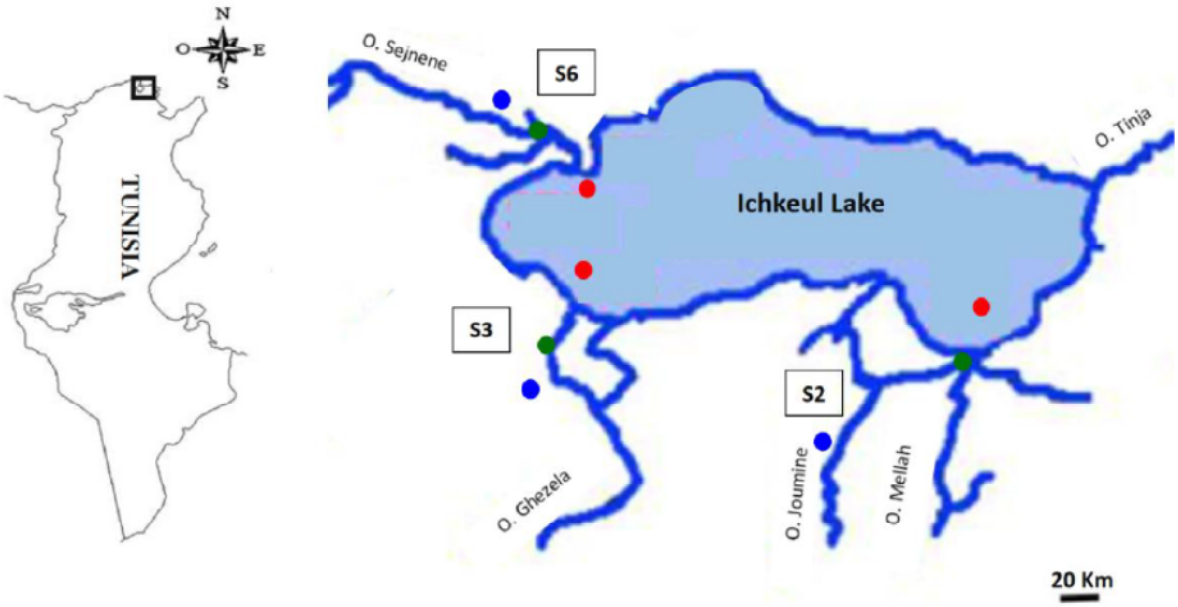

Fig. 1. Localisation of the sampling stations S2, S3 and S6 located around the Ichkeul Lake (Tunisia). At each station a continuum consisting of agricultural soil (bleu circles), river sediment (green circles), and lake sediments (red circles) was sampled. O. Oued (wadi) followed by the river name.

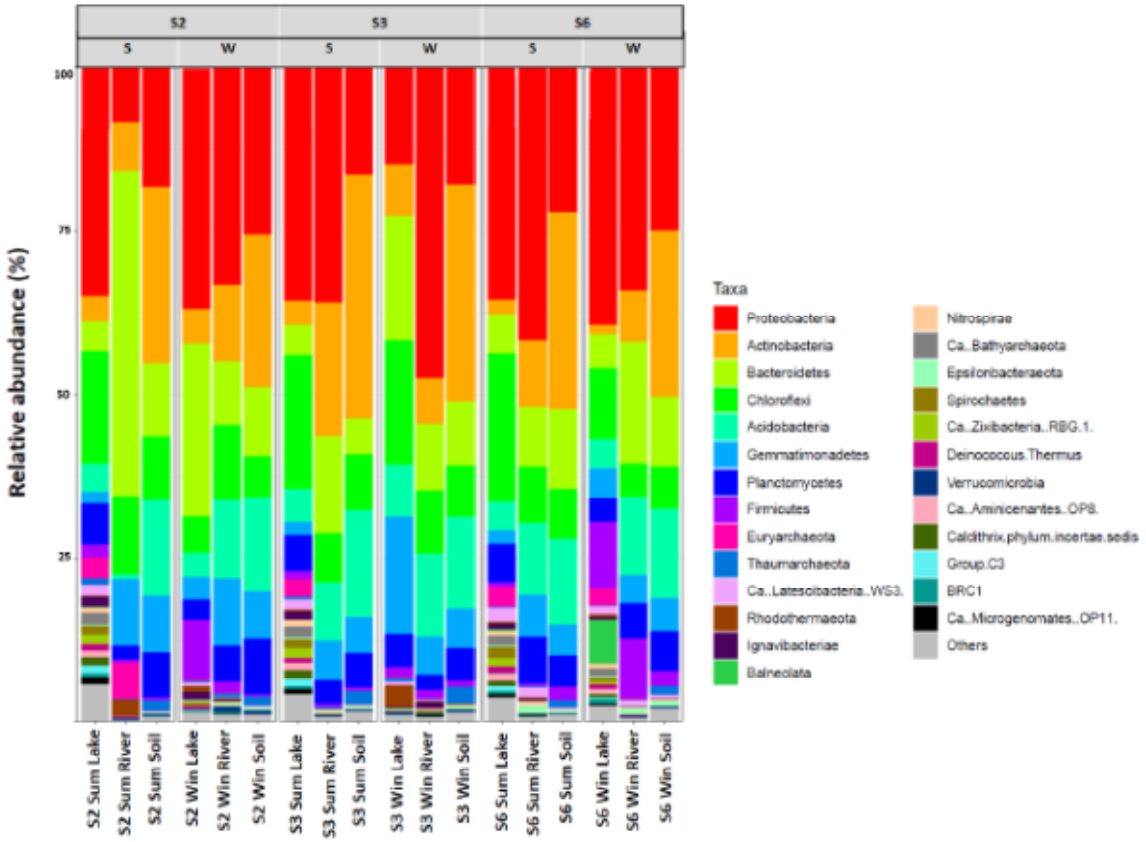

Fig. 2. Bacterial community compositions at the phylum level along the continuum sites (soil, river and lake) at each station (S2, S3 and S6) in summer (S) and winter (W). The bar plots represent the mean of three replicates. Other, phyla with relative abundance < 5%.

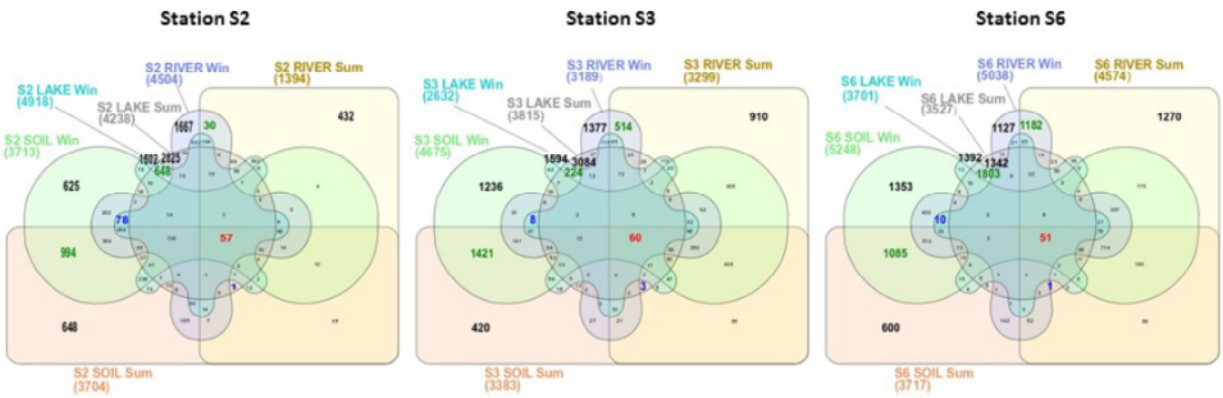

Fig. 3. Comparison of bacterial communities in the different Stations (S2, S3, and S6). The Venn diagram shows the number of specific OTUs (black bold) for each site (soil, river and lake) in winter (Win) and summer (Sum), and the core communities corresponding to the number of shared OTUs between all sites either at both seasons (red) or at each season (blue). The number of shared OTUs between the seasons for each site is highlighted in green.

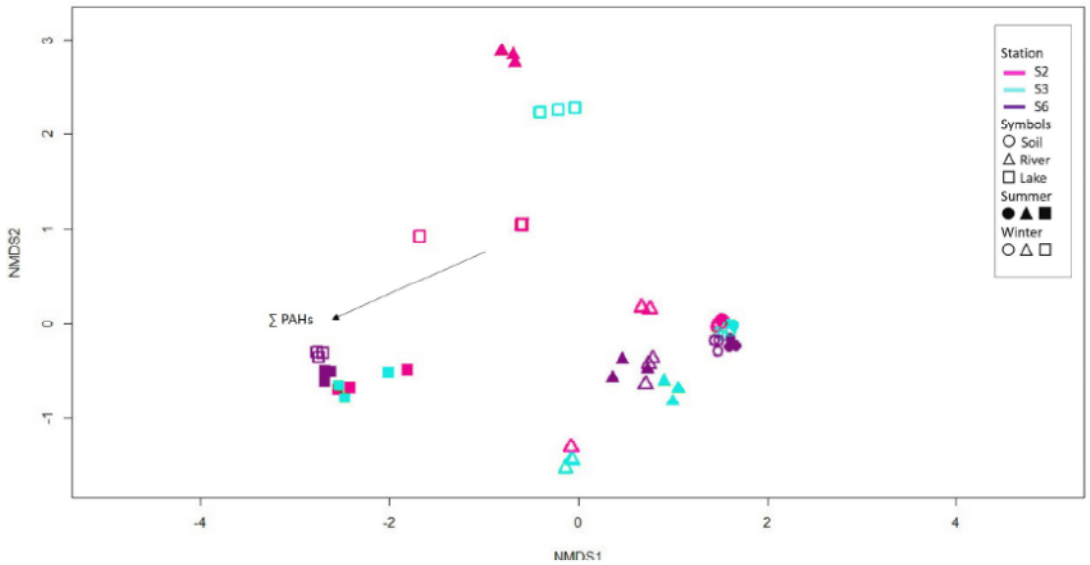

Fig. 4. Comparison of bacterial communities inhabiting the continuum (soil, river and lake) at each station (S2, S3 and S6) in summer and winter by non-metric multidimensional scaling (NMDS). The arrow represents the significant environmental variable (∑ PAHs; $p < 0.07$) fitted as a vector using the envfit function. 2D stress: 0.095.

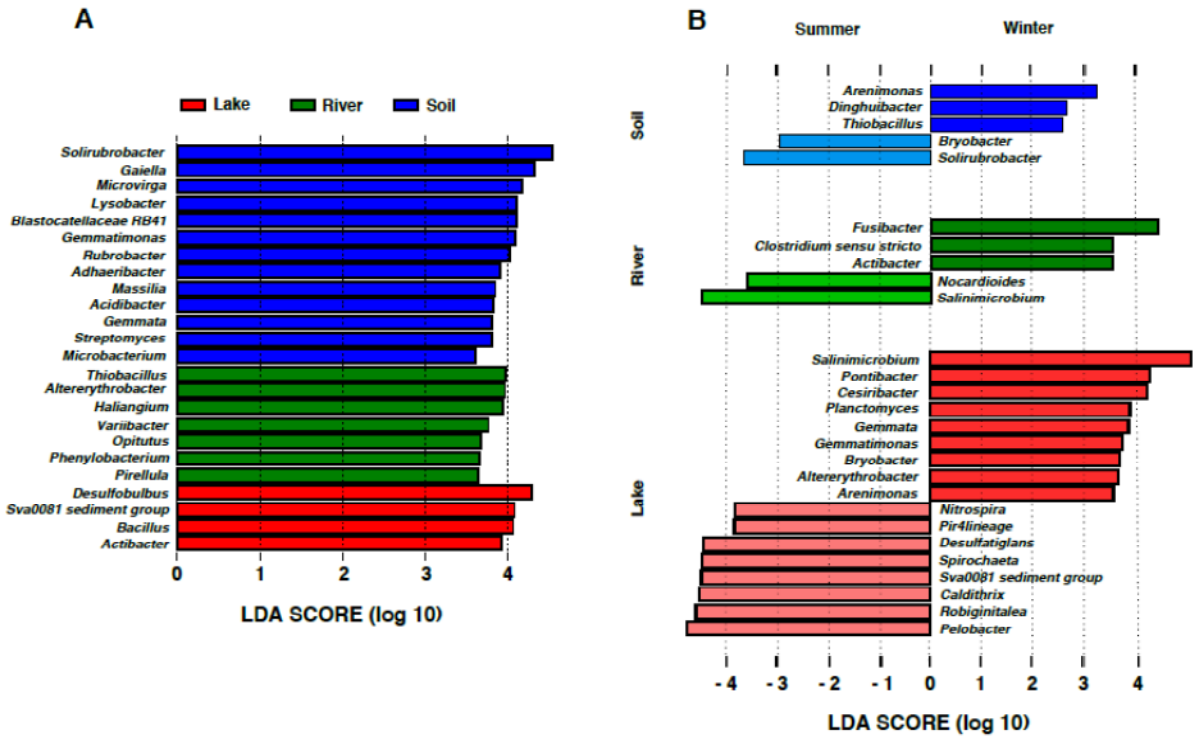

Fig. 5. Comparison of bacterial communities from the stations located around the Ichkeul Lake. Linear discriminant analysis effect size (LEfSe) comparing (A) the compartments (soil, river and lake) and (B) the season (summer and winter) within each compartment.

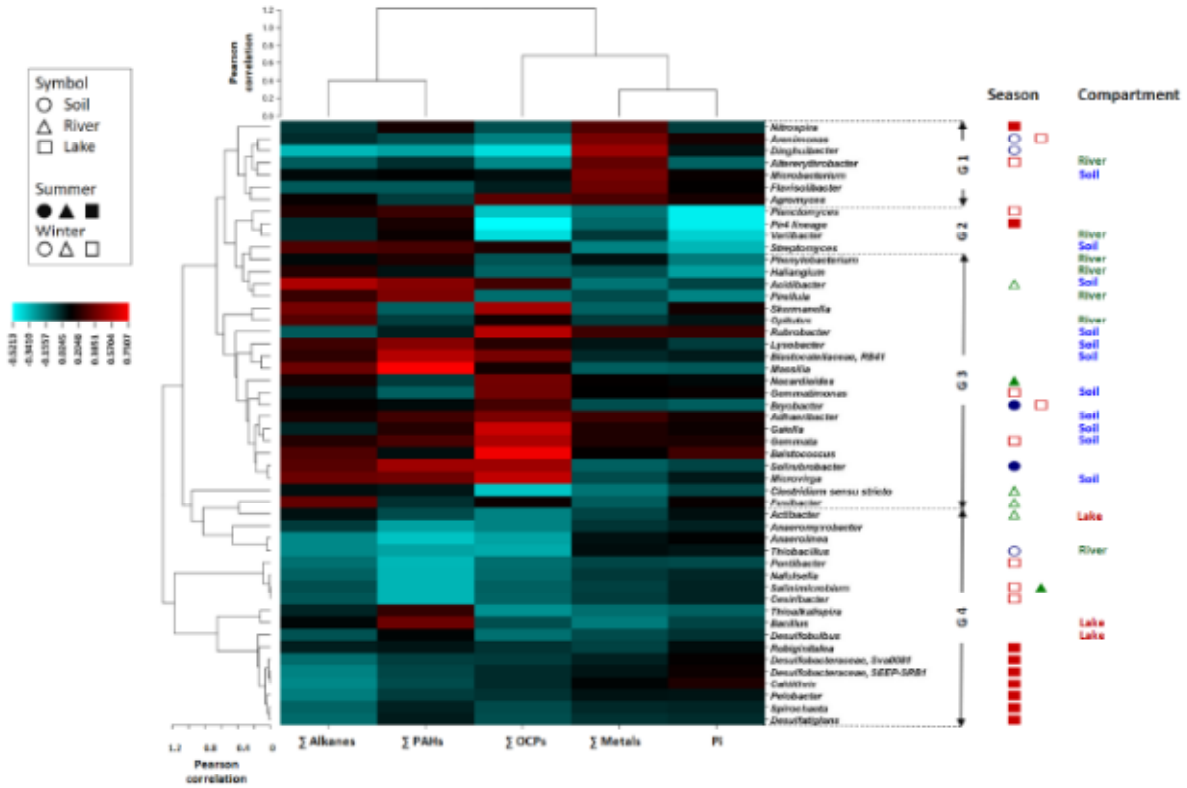

Fig. 6. Correlation between bacterial OTUs and pollutant concentrations by clustered correlation (ClusCor) analysis. The analysis is based on Pearson correlation, red indicating high positive correlation and blue high negative correlation (p value < 0.01). Clustering was performed applying Pearson correlations and Ward algorithm as cluster method. 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 according to compartment: soil (blue circle), river (green triangle) and lake (red square); or the season: summer (full symbol) and winter (empty symbol). Σ Alkanes, sum of alkane compounds; Σ PAHs, sum of polyaromatic hydrocarbons; Σ OCPs, sum of organochlorine pesticides; Σ metals, sum of metals; Pi, pressure index.

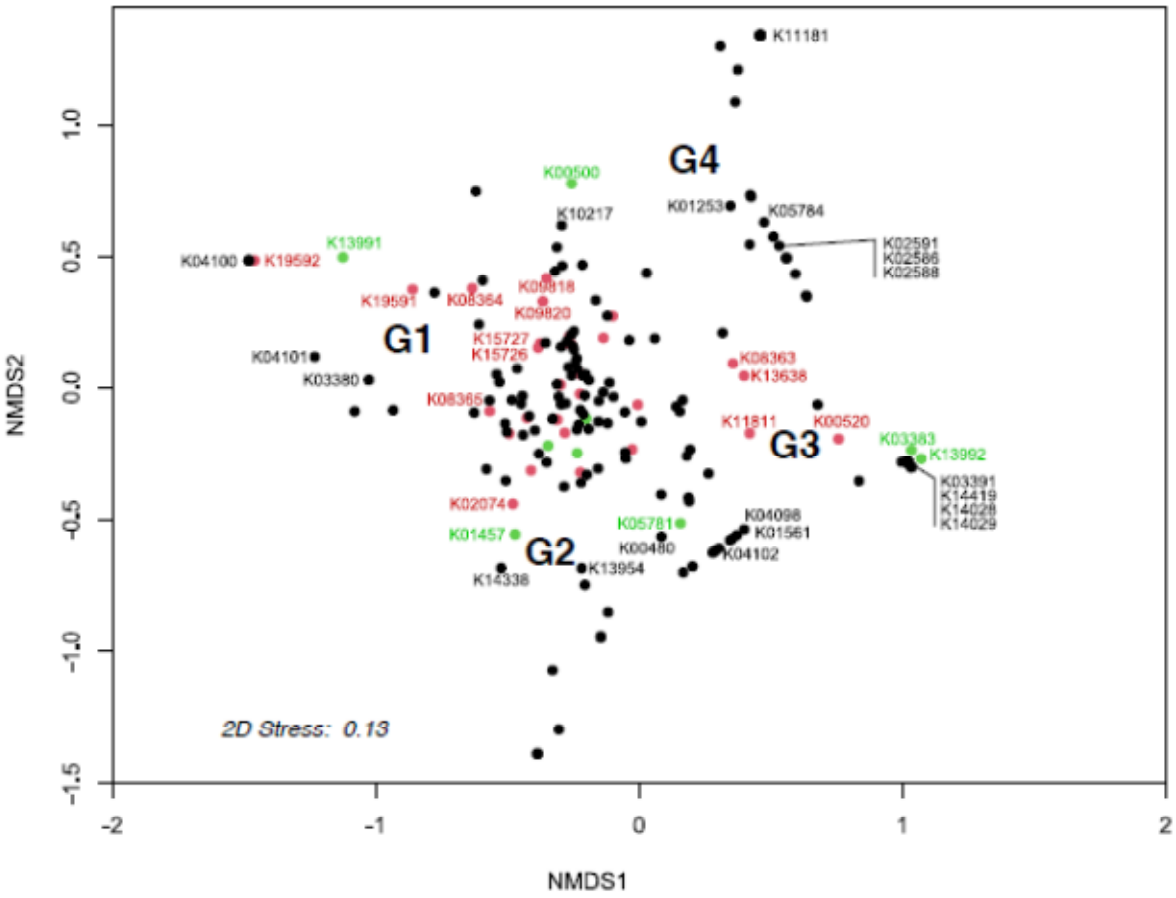

Fig. 7. Comparison of PICRUSt-predicted pollutant degradation/transformation capabilities of the genera groups (G1-G4). The genera groups (G1-G4) identified by the ClusCor analysis (Fig. 6) were compared by NMDS based on PICRUSt-predicted genes involved in pollutant degradation/transformation, including metals (red), pesticides (green), and hydrocarbons (black). The most relevant genes specifically abundant in each genera group discussed in the text are identified by numbers and their description provided in supplementary material Table S2.