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- 1 Functional redundancy in response to runoff input upholds microbial community in
- 2 hydrocarbon-contaminated land-sea continuum
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12 Abstract

An experimental approach mimicking the land-sea continuum in microcosms was developed in order to determine the effect of the terrigenous inputs by soil runoff on the microbial functional potential in hydrocarbon (HC) contaminated marine coastal sediment. We hypothesized that the coalescent event increases the functional potential of microbial communities in marine coastal sediments, influencing the fate of HC in marine coastal ecosystems.

19 The microbial functional potential including the HC degradation ability was assessed by 20 DNA-array to compare the sediment receiving or not terrigenous inputs. The removal of HC 21 and the functional gene richness in sediment was unchanged with the terrigenous inputs. 22 However, the gene variants (GVs) composition was modified indicating functional 23 redundancy. In addition, functional indicators including GVs related to sulfite reduction, 24 denitrification and polyaromatic degradation were identified in higher proportion in sediment 25 receiving terrigenous inputs. The terrigenous inputs modified the functional co-occurence 26 networks, showing a reorganization of the GVs associations with an increase of the network 27 complexity. Different keystone GVs ensuring similar functions were identified in networks 28 with or without terrigenous inputs, further confirming functional redundancy.

We argue that functional redundancy maintains the structure of microbial community in hydrocarbon-contaminated land-sea continuum mixing zone. Our results provide helpful functional information for the monitoring and management of coastal environment affected by human land-based activities.

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Keywords: functional gene array, bioindicators, runoff, terrigenous inputs, marine sediment,
 hydrocarbon degradation

37 1. Introduction

38 Human activities threaten the ecosystem services of coastal areas by introducing contaminants (Halpern et al., 2008). Among these contaminants, petroleum hydrocarbons 39 40 (HC) are of major concern because of their toxicity. Coastal areas represent a land-sea 41 continuum, mixing terrestrial and marine ecosystems (Laruelle et al., 2013; Xenopoulos et 42 al., 2017). Soil runoff drains terrigenous inputs to marine coastal sediments including organic 43 matter, sorbed pollutants and microorganisms (Fagervold et al., 2014; Hoffman et al., 1984; 44 Yakirevich et al., 2013). The composition of terrigenous inputs is influenced by the vegetation 45 cover, which alters water flow (Nagase and Dunnett, 2012; Wenzel, 2009) and regulates the 46 soil microbial communities. The mixing of microbial communities together with the mixing of 47 their environments results in community coalescence (Châtillon et al., 2023; Rillig et al., 2015). The coalescence events raise questions about the environmental and biotic 48 49 processes that structure microbial communities, particularly regarding the functional aspect 50 (Rillig et al., 2016; Rocca et al., 2020). Although several studies have investigated functions 51 considering coalescent communities in agriculture, industry and medicine (Calderón et al., 52 2017; Sierocinski et al., 2017; Wilson et al., 2019), the functional aspect is still unclear in 53 aquatic ecosystems yet limited to some enzymatic activities (Castledine et al., 2020; Mobilian 54 et al., 2020). Regarding HC, the functional potential of degradation has been largely studied. independently in soil (Patel et al., 2016) and in marine coastal environment (King et al., 55 2015), revealing functional genes as markers of HC degradation (e.g. von Netzer et al., 56 57 2016), but information in the context of coalescence is lacking. However, the microbial 58 communities inhabiting the land-sea continuum play a pivotal role in determining the fate of 59 pollutants (Bauer et al., 2013; Duran and Cravo-Laureau, 2016), involving microbial network 60 interactions, which are probably affected by terrigenous inputs (Yan et al., 2021). It has been 61 shown that different metabolic groups, ensuring not only degradation functions but also 62 involved in biogeochemical cycles (Roling and van Bodegom, 2014), compose HC degrading 63 microbial communities. It is thus of paramount importance to investigate the functional

64 component of those communities in order to have a comprehensive view of the functional 65 network in a coalescence context. In order to assess the microbial functionality, different approaches have been developed including measurements of fluxes and activities (Ziervogel 66 67 et al., 2016), inference from 16S rRNA gene barcoding (Douglas et al., 2020), targeting of functional genes (Duarte et al., 2014), and meta-omics (Mason et al., 2012). Recently, DNA-68 69 array approach has been shown to assess the microbial functionality with the advantages to 70 target both structure and functions of microbial communities in various environments (Shi et 71 al., 2019). DNA-array provides the opportunity to follow easily the microbial community 72 functional dynamics that could be useful to monitor the effect of coalescence event. This 73 approach, used in environmental continuum, has provided contrasted results showing either 74 the stability (Avila-Jimenez et al., 2020) or the vulnerability (Yan et al., 2021) of the functional 75 microbial network. To the best of our knowledge, such studies on the functional diversity and 76 the functional networks are still missing within the land-sea continuum. There is thus need to 77 study the effect of the multiple coalescent events on the microbial functional potential and the 78 functional interactions in marine coastal sediments receiving terrigenous inputs. In order to 79 determine the effect of coalescent event on the functional diversity and the functional 80 interactions in the land-sea continuum, an experimental ecology approach, simulating the 81 continuum, was developed. This microcosm experiment was designed to consider the 82 coalescence events and the effect of plant and HC influencing the runoff. This approach 83 allows the implementation of artificial scenarios by controlling physical-chemical parameters 84 mimicking as close as possible natural environmental conditions (Cravo-Laureau and Duran, 85 2014; Mazière et al, 2023). We hypothesized that terrigenous inputs increase the functional potential of microbial communities in marine coastal sediments and thus influence the fate of 86 87 HC in coastal ecosystems. DNA-array (Geochip 5S) gene functional analysis was performed 88 to compare microbial communities in sediment receiving or not terrigenous inputs. Different 89 gene families, involved in biogeochemical cycles and organic pollutant remediation, were targeted. Functional genes involved in HC degradation pathways were examined as 90

91 candidate indicators of the community coalescence. Their co-occurrence patterns with92 functional genes involved in the main biogeochemical cycles were established.

93

94 2. Material and Methods

95 2.1. Microcosms

96 Soil (10 kg) and sediment (2 kg) were collected in the same geographical area, close to 97 petroleum refinery, in southeast of France, in October 2017. Soil, classified as peyrosol 98 (gravelly soil, Baize et al, 2008; Messant et al, 2021), was collected with manual auger from 99 the surroundings of the Berre Pond (43°29'6.9"N 5°11'18.53"E, France) and sediment from 100 the Canal Viel Cove (43°23'20.3"N 4°59'31.8"E, France). The sampling sites are both near 101 industrial zones, contaminated by HC and enlisted int the BASOL database (Antoni, 2013) 102 for the polluted sites and soils from the French Ministry of the Ecological and Solidary 103 Transition. Soil, rid of vegetation, was collected on surface (maximal depth of 15 cm) and 104 sediment was collected from the surface until 8 to 10 cm depth. Prior to microcosms setup, 105 soil and sediment were sieved to < 4 mm and < 1 mm respectively, to remove coarse 106 gravels, plant debris and macroorganisms, and to homogenise samples. Soil was air-dried 107 and stored at room temperature and sediment was stored at 4 °C in a 5 L bottle completely 108 covered with in situ seawater, until starting the experiment (one week later). The seawater 109 covering the sediment was submitted to continuous aeration through an aquarium pump. The 110 texture, the pH, the C/N ratio, the cation-exchange capacity, the water holding capacity 111 (WHC), ions and trace metals were quantified on dry soil and sediment (LAS, INRAE Arras, 112 France). Microcosms were set up and incubated for 39 days under controlled conditions, as 113 previously described (Châtillon et al., 2023). The experimental system was designed to 114 highlight community coalescence between soil and coastal marine sediments resulting from 115 terrigenous inputs via soil runoff, considering the effect of plant and HC influencing 116 potentially the runoff. Microcosms were set up in triplicates as follow (Figure 1): land-sea 117 continuum microcosms that simulated the soil runoff to marine coastal sediment connecting

soil and sediment compartments, and control microcosms without runoff of soil consisting ofsediment compartment.

Briefly, the microcosms were composed of glass devices (1 L beaker equipped with a 120 121 seawater inlet and outlet) containing the equivalent of 90 g of dry sediment and that were 122 connected through Teflon pipes or not to stainless steel devices containing 600 g of 123 moistened soil at 60% WHC. Different conditions were applied in triplicate (Figure 1) 124 combining the presence of plant (P) or not (NP) in soil with the spiking of deuterated HC (C) 125 or not (NC) in soil and sediment. The grass Catapodium marinum (seed bank of the Muséum 126 National d'Histoire Naturelle de Paris - DGD – MJZ) was used and after seed germination 16 127 plants were kept per devices. In the HC spiked conditions, soil and sediment (corresponding 128 to 1/10 of the soil and sediment in the devices) were mixed to 1:1 (W:W) of deuterated n-129 hexadecane (D34, 98%, Cambridge Isotope Laboratories, Inc, USA) and phenanthrene 130 (D10, 98%, euriso-top, France), dissolved in hexane solvent. A stock solution was prepared 131 in order to add each HC at a concentration of 100 mg/kg dry soil or sediment. The HC 132 content in soil and sediment before spiking was between 0.8 and 1.8 mg/kg. For the 133 unspiked conditions, the same volume of hexane was mixed to sediment and soil, without 134 addition of HC stock solution. In land-sea continuum microcosms, soils were watered to 135 reach 60% of their WHC and were daily watered by means of a syringe dispensing 73 ml 136 mineral water (Volvic®) to mimic rainfall and produce runoff waters. In both land-sea 137 continuum and control microcosms, peristaltic pumps helped to simulate tidal cycles and 138 renew the seawater (Salt Instant Ocean, Aquarium systems) ensuring conditions close to 139 those prevailing in natural environment. Two tidal cycles were simulated per day: high tide 140 was simulated filling the sediment compartments with 250 mL of synthetic seawater in 6 141 hours, and low tide was simulated reducing water level in 6h, maintaining sediment covered 142 by 3 cm of seawater.

143

144 2.2. Sampling

145 Samples of the different compartments (soil, runoff, sediment) and of seawater were collected at different times at the beginning (t0) at the end (tf, after 39 days of incubation), 146 147 and during the incubation (after 23 days of incubation) for subsequent chemical and/or 148 biomolecular analysis. Soil and sediment samples (ca. 10-12 g) were collected in triplicates 149 at the beginning and the end of the experiment (t0 and tf respectively) for the DNA extraction 150 and the Geochip analysis. Soil samples were collected using a little stainless-steel corer 151 (diameter: 14 mm; height: 40 mm; Bourceret et al., 2015) and sediment samples using a 152 spoon. At t0, three soil samples (each being the mixture of 3 cores) were collected from soil 153 microcosm specifically set up for this sampling time, one with HC spiking and one without HC 154 spiking, in order to do not alter soil structure or disturb plant germination in the microcosm to 155 be incubated longer. Three sediment samples (each being the mix of 3 spoons) were 156 collected from the sediment batch prepared for sediment devices with HC spiking (NPC, PC, 157 NRC conditions) and from the one prepared for sediment devices without HC spiking (NPNC, 158 PNC, NRNC conditions). After 23 days of incubation and at tf (39 days), runoff samples were 159 obtained from each land-sea microcosm, by mixing runoff from three consecutive days and 160 filtered on 0.22 µm membrane (Millex). An aliquot of Volvic water (rainwater) was filtered in 161 the same way as the runoff to serve as a control. At tf (after 39 days of incubation), 162 Catapodium marinum were cut at the collar, put in paper bags, dried and weighted to 163 estimate the aerial dry biomass. Soil samples were collected in triplicate from each land-sea 164 continuum microcosm using a template (three notches per row and three per column) as a 165 guide for the corer. The cores from the same column were mixed and formed one sample. 166 Sediment samples were collected from each microcosm in triplicate after mixing of sediment 167 using a spoon. Thus, the sampling design provided three technical replicates for each of the 168 three biological replicates, for soil and sediment from each microcosm. A portion of the soil 169 and sediment samples were dedicated to chemical analyses and were stored in glass tubes 170 (supelco) at -20°C while that dedicated to biomolecular analyses was stored at -80°C.

172 2.3. Quantification of the dissolved organic carbon (DOC) and deuterated HC

173 Runoff water (40 mL) and seawater from the low tides (250 mL) were collected at the 174 beginning, after 23 and 39 (tf) days of incubation and filtered on GF/F filters. 20 mL were 175 kept at -20°C until DOC analyses. DOC was measured using TOC-VCSH Analyzer 176 (Shimadzu, Japan) with ASI-V switcher. Freeze-dried soil and sediment samples (2 g) 177 collected at the beginning (t0) and at the end of the experiment (tf) were sequentially 178 extracted with dichloromethane (DCM) followed by methanol by accelerated solvent 179 extraction (Dionex, ASE350). ASE cells were filled with activated copper powder (to remove 180 molecular sulfur) and sodium sulfate (Na₂SO₄) to remove remaining water. Samples were 181 extracted at 130 °C, 100 bars and 10 min for each solvent. After adjusting the volume of the 182 DCM extract at 10 mL, an aliquot of 80 µL was spiked with 20 µL of internal quantifications 183 standards (Fluorene D10 and Fluoranthene D10) to quantify deuterated HC (phenanthrene 184 and n-hexadecane) and injected by gas chromatography-mass spectrometry (GC-MS. The 185 instrument used was an Agilent 7820 gas chromatograph equipped with a DB 5-MS column 186 (60 m × 0.25 mm i.d. × 0.25 µm film thickness) coupled with an Agilent 5975 mass selective 187 detector operating in single ion monitoring mode. The molecules were detected with a 188 quadrupole analyzer following ionization by electronic impact. The temperature program was 189 the following: from 70 to 130 °C at 15 °C min⁻¹, then from 130 to 315 °C at 3 °C min⁻¹ and 190 then a 15 min hold at 315 °C. One microliter of sample was injected in splitless mode at 300 191 °C. The carrier gas was helium at 1.4 mL min⁻¹ constant flow. The quantitative analyses of 192 PACs were carried out using internal calibration. For each quantified compound, the GC/MS 193 was calibrated between 0.06 and 9.6 µg mL⁻¹ with 10 calibration levels. The calibration 194 curves were drawn and satisfactory determination coefficients were obtained (r^2 >0.99). To 195 verify the quantification, two calibration controls (lower and higher concentrations) were 196 carried out every six samples and only a deviation lower than 20% was accepted.

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198 2.4. DNA preparation, functional gene analysis and bacterial abundance

199 DNA was extracted using the DNA PowerSoil Isolation Kit (MoBio Laboratories) on 0.25 g for 200 soil and sediment samples or on the whole 0.22 µm filter for runoff and rainwater samples. 201 Extractions were performed according to the manufacturer's instructions with a slight 202 modification: samples were ground in liquid nitrogen as prior step. DNA concentration was 203 estimated using Qubit dosage and DNA quality was investigated by spectrophotometry and 204 with agarose gel electrophoresis. DNA extracts were stored at -20°C. GeoChip 5.0S 205 (Glomics Inc., Norman, OK, United States), manufactured by Agilent Technologies (Santa 206 Clara, CA, USA), was used to detect specifically, sensitively and quantitatively key functional 207 genes from soil and sediment microbial communities at the end of the experiment. The DNA-208 array contains ~57,000 probes covering ~151,000 target genes of 1,517 gene families from 209 functional categories involved in biogeochemical cycling of C, N, S, and P and in other 210 ecological services such as organic remediation (Shi et al., 2019). The probes were designed 211 to detect a single sequence or a similar group of sequences, called a gene variant (GV), 212 corresponding to a target functional gene present in the NCBI databases. Glomics conducted 213 the DNA-array analysis, which consisted of amplification (Wu et al., 2006), labelling 214 hybridization and data pre-processing (normalization and quality filtering based on the 215 average signal intensity of internal standards and the average background signal) (Van 216 Nostrand et al., 2016) from an aliquot of the DNA extracts. Pre-processed data were loaded 217 under the free software R (R Core Team, 2020) and treated using phyloseg package 218 (McMurdie and Holmes, 2013). Prior to statistical analyses, signals were converted into 219 relative abundances. The bacterial abundance was estimated in runoff and rainwater (at t1 220 and t2) and in soil and sediment (tf), using quantitative real time PCR to quantify the 16S 221 rRNA gene copy number. The qPCR assay was performed as described in Cébron et al. 222 (2008) by using the primer set 968F/1401R (Felske Andreas et al., 1998) and iQ SYBR 223 green SuperMix (Bio-Rad). Absolute quantification was assessed using a CFX96 Real-Time 224 PCR detection system (Bio-Rad, France) and a 10-fold dilution series from 10⁸ to 10¹ copies 225 μ I⁻¹ of the standard plasmid. Data were then expressed as gene copy numbers per gram of 226 dry weight soil/sediment or per mL of runoff.

227

228 2.5. Statistical analysis

229 All the statistical analyses were performed within the R programming environment (R Core 230 Team, 2020). The Hill numbers were calculated at three different orders (q) of diversity with a 231 q value of 0 for species richness with all species having the same weight, q = 1 for the 232 exponential of Shannon's index with species being weighted exactly for their abundance in 233 the community and whereas q = 2 for the inverse-Simpson's index which favours abundant 234 species. The Simpson's Evenness was calculated as the inverse-Simpson divided by 235 species richness. Analysis of variance (ANOVA) was implemented to determine significant 236 alpha-diversity differences between microbial communities under the different conditions. 237 When the overall ANOVA was statistically significant, the Tukey post hoc test was used to 238 identify statistically significant pairwise differences between communities. Differences in 239 functional gene composition between communities was quantified using Bray-Curtis dissimilarity and further visualized using non-metric multidimensional scaling ordinations 240 241 (NMDS). To assess the sources of variation (i.e. compartment, HC spiking, presence of 242 plants, runoff) in the functional dissimilarity matrix, a series of permutation multivariate 243 analyses of variance (PERMANOVA; Anderson, 2001) was carried using permutation tests 244 with 9,999 iterations with the function adonis in the R package vegan (Oksanen et al., 2019). 245 Post-hoc pairwise comparisons were performed using the package RVAideMemoire (Hervé, 246 2020). A procrustes analyses was performed to determine the overall degree of concordance 247 between dissimilarity in all GVs and dissimilarity only remediation category GVs. In the 248 Procruste analysis, a pair of data matrices (i.e. the scores of the communities from two 249 NMDS generated from dissimilarity matrices) is compared by using a rotational-fit algorithm 250 that minimizes the sum of squared residuals between the two matrices. Concordance was 251 then quantified with a correlation index in the symmetric Procrustes rotation. P-values were 252 evaluated with 9999 Monte Carlo permutation tests. In order to identify gene indicators, a 253 multi-level pattern analysis (MLPA) was conducted using point-biserial correlation coefficient

254 (De Cáceres et al., 2010), suitable to DNA-array abundance data. MLPA calculates the 255 association index of a feature (i.e. gene family or GVs) with each particular condition and 256 other combinations of conditions to infer the best association (highest association index 257 value revealing the indicator). The statistical significance of the association index was tested 258 by Monte Carlo method. This correlation index assesses the positive or negative preference 259 of features for the conditions or combinations of conditions compared to the other ones. 260 MLPA was led at the gene family and GVs levels, using the R package indicspecies 261 (Caceres and Legendre, 2009). Combinations of conditions were restricted to those with a 262 biological meaning. ANOVAs were also implemented to test the effect of the experimental 263 conditions (i.e. compartment, HC spiking, presence of plants, runoff) on the bacterial 264 abundance, the aerial vegetal dry weight, and the DOC content. Non-parametric Wilcox test 265 and Kruskall-Wallis tests were used to test the effect of the experimental conditions on the 266 removal of the deuterated HC.

267

268 2.6. Functional co-occurrence network inference

269 The network analysis was computed with the application CoNet in Cytoscape (Faust et al., 270 2015; Faust and Raes, 2016). The significant co-presence and mutual exclusion patterns 271 were detected depending on the runoff among the 873 GVs identified as indicators as 272 specified above. Briefly, GVs were filtered with the fewest occurrence (<10) and counts were 273 transformed as relative abundance. Multiple associations were determined by combining 274 correlations (Pearson and Spearman) and dissimilarities (Bray-Curtis and Kullback Leibler) 275 with an initial automatically threshold set, such as the initial network included 1,000 positive 276 and 1,000 negative edges for each coefficient. The significance of edges was assessed by 277 merging permutation and bootstrap distributions (pvalues <0.05, Brown's method) combined 278 with multiple-testing correction (Benjamini and Hochberg's procedure). Modules (highly 279 interconnected nodes) and keynodes in networks were identified using the application 280 MCODE (Molecular Complex Detection) in Cytoscape (Bader and Hogue, 2003).

281

282 3. Results and Discussion

3.1. HC removal and impact on the plant biomass and the bacterial abundance

284 The concentrations of the deuterated HC at the beginning of the experiment (t0) and after 39 285 days of incubation (tf) were determined in the different microcosms (Table 1). The phenanthrene concentration was reduced significantly in soil (up to 95 %) and in sediment 286 287 (around 68 %) samples, whereas hexadecane removal was observed only in soil samples (up to 29 %; Wilcoxon test, p < 0.05). The phenanthrene removal was not influenced neither 288 by the presence of plant in soil (Wilcoxon test W = 4; p = 1) nor by the terrigenous inputs in 289 290 sediment (Kruskall-Wallis test: H =1.69, p = 0.43, df = 2). The HC removal was higher in soil 291 than in sediment (Wilcoxon tests: W = 54; p < 0.0001). It was surprising to observe a higher 292 removal of phenanthrene compared to hexadecane because hexadecane is considered as 293 less toxic and more easily used by a wide range of microorganisms (Van Hamme et al., 294 2003; Leahy and Colwell, 1990; Yemashova et al., 2007). However, after a contamination, 295 microbial communities able to degrade aromatic compounds could dominate before those 296 able to degrade alkane in coastal sediment, as described during the Deepwater Horizon 297 disaster (for a review, see Kimes et al., 2014). Such capacity might be explained by the fact 298 that microbial communities were probably adapted to the presence of aromatics compounds 299 (legacy effects; Jurburg et al., 2017; Nunes et al., 2018; Thomas et al., 2020) and the 300 bioavailability of the compound (Ghosal et al., 2016). In contrast to that previously reported 301 (Bourceret et al., 2016; Khan et al., 2013; Rohrbacher and St-Arnaud, 2016), the presence of 302 plants did not influence the HC removal. Plant biomass and prokaryotic abundance (Table 303 S1) were estimated to determine the ecotoxicological effect of the deuterated HC spiking in 304 microcosms. The aerial vegetal dry weight was not significantly different between the non-305 spiked soil (Soil NC) and the HC spiked soil (Soil C) conditions (Wilcox test: W = 1, p = 0.2) 306 showing that Catapodium marinum was not sensitive to HC contamination. To our 307 knowledge, there is no available information about the effect of the HC on this grass species.

308 However, grasses such as perennial ryegrass or tall fescue are often used in HC phytoremediation (Cook and Hesterberg, 2013) showing enhanced HC removal efficiency 309 310 (Yavari et al., 2015). The prokaryotic abundance was of the same order of magnitude in the 311 different soil and sediment compartments (around 4.6x10⁸ 16S rRNA gene copies per g DW), 312 irrespective of the condition (ANOVA within soil samples: F = 1.05, p = 0.42; ANOVA within 313 sediment samples: F = 0.4, p = 0.84). Unexpectedly, we did not observe an effect of the 314 plants on the prokaryotic abundance although it has been shown that plants provide 315 substrates and nutrients for microbial growth (Merkl et al. 2004; Inckot et al. 2011; Smith et 316 al. 2006; Shirdam et al. 2008; Hinsinger et al. 2005). Significant lower prokaryotic abundance was found in runoff samples (2.8x10³ 16S rRNA gene copies per mL; ANOVA; F=103.63; p < 317 318 0.001) than in soils and sediments, but higher (X 100) than in rainwater controls showing that 319 runoff is enriched with prokaryotic abundance as it passes through the soil. Similarly, runoff 320 was also enriched with DOC irrespective of the condition (Table S2) showing it brings 321 microorganisms and organic matter to the marine compartment, as it was previously 322 described (Le et al., 2016). Surprisingly, the presence of plant did not change the DOC 323 content contrary to previous report (Janeau et al., 2014). During our experiment, more DOC 324 was found in the seawater receiving runoff (Sed R; ANOVA, F=18.150, p < 0.001) than that 325 from control microcosm without runoff (Sed NR). Such observation showed DOC enrichment 326 in the marine compartment, which was even more important in the non-spiked seawater (Sed 327 NC) (ANOVA, interaction runoff: HC; ANOVA, F=6.534; p < 0.05). Such observation was 328 consistent with previous studies indicating that DOC and HC establish complex interactions 329 in soil (Ukalska-Jaruga et al., 2019) influencing the DOC composition in runoff (Daryabeigi 330 Zand, 2020; Janeau et al., 2014), which in turn influence the microbial community structure 331 and activity (Giorgio and Davis, 2003; Le et al., 2016; Pommier et al., 2014; Tian et al., 332 2015). In order to reveal the effect of the runoff on microbial community in marine 333 compartment, the microbial functional capabilities were assessed by DNA-array analysis.

334

335 **3.2.** Diversity, similarity and unique gene sequence variants

336 The functional gene diversity and composition (non-expressional DNA-array analysis) was 337 explored on soil and sediment from the different microcosms after 39 days. A total of 51 897 338 gene variants representing 90 % of the 57 498 unique sequences (Shi et al., 2019) were 339 detected on the GeoChip 5.0 S. The minimum GVs was found in sediments receiving runoff 340 (SedR) from planted and HC spiked soils (Sed R PC; 32,274 ± 1751; Table 2) whereas the 341 maximum GVs was found in sediments receiving runoff from non-planted and HC spiked 342 soils (SedR NPC; 45,641 \pm 1277; Table 2). The coverage was between 56 % \pm 3.0 and 79 % 343 ± 2.2 (Table 2). The GVs richness was significantly higher in sediment than in soil 344 (Exponential Shannon 1D: p < 0.05; Table S3) indicating a prevalence of abundant GVs in 345 sediment, which can be explained by the properties of matrices. However, the relative 346 abundances of main functional gene categories (carbon cycling, metal homeostasis, 347 nitrogen, organic remediation, phosphorus, sulfur, secondary metabolism, virulence, and 348 other) were similar (Figure S1) as previously observed in marine coastal environment 349 (Kappell et al., 2014). Such observation was also consistent with previous studies showing 350 that sediments and soils harbour the same functions but with specific pathways (for review, 351 Fenchel et al., 2012a, 2012b). No significant difference of GV richness was found within soils 352 irrespective of the condition indicating that neither plants nor the HC spiking changed the 353 functional diversity. However, the GVs richness was significantly different in planted soil with 354 HC spiking (richness R: p < 0.05; Table S3) indicating that the interaction of the plants and 355 the HC spiking affected the microbial functional diversity in soils. In sediments, the GVs 356 richness was significantly higher in sediments receiving runoff from non-planted soil (SedR 357 NP) (richness R: p<0.01; Table S3) than in sediments receiving runoff from planted soil 358 (SedR P). It is likely that the runoff from planted soils exerted a selection on the functional 359 gene diversity in sediments. The HC spiking had no effect on the GVs richness in sediments 360 as observed in soils confirming that microbial communities were adapted to the presence of 361 HC.

362 To further characterize the effect of runoff on the functional organization, the GVs 363 composition was investigated. The relative abundance of functional gene categories (Figure 364 S1) observed for all conditions revealed the functional stability of microbial communities. 365 However, the comparison of functional microbial communities based on the GVs from all 366 gene categories (Figure 2 A) showed similar pattern than that obtained by comparing the 367 functional microbial communities based on the GVSs involved in the organic remediation 368 (Figure 2 B), which was further supported by Procrustes ($M^2 = 0.017$, r = 0.99, p < 0.001). 369 Such observation suggested that the presence of HC, in the soil and sediment contaminated 370 for a long period might explain the functional organization of the microbial communities 371 because they are likely adapted to the presence of HC. Thus, HC probably act as a filter 372 selecting the organisms that are able to survive and establish in this specific condition 373 (Bordenave et al., 2004; Duran et al., 2015; Stauffert et al., 2013). However, the functional microbial communities structure was not affected by the spiking of HC (PERMANOVA F(1) = 374 375 1.0825; p > 0.05) suggesting a legacy effect (Jurburg et al., 2017; Nunes et al., 2018; Hafez 376 et al, 2022). Additionally, NMDS analysis separated clusters according to the compartment, 377 distinguishing the effect of runoff in sediments (Figure 2), which indicated functional 378 redundancy, similar function carried by different genes and/or species (Jurburg and Salles, 379 2015; Louca et al., 2018), as illustrated in Figure 2 B for the organic remediation function. 380 PERMANOVA confirmed soil and sediment separate clustering (F(1) = 3.2066; p < 0.05) 381 unveiling that the ecological niches are the main filter for the organization of the function of 382 the communities as previously proposed (Tringe et al., 2005). Among soil samples, the presence of plant did not affect the functional potential (PERMANOVA F(1) = 1.1077; p > 383 384 0.05). This result was not expected because several studies reported that microbial 385 communities and even specific gene content are affected by the roots (Bourceret et al., 2016; 386 Cébron et al., 2009; Gerhardt et al., 2009; Newman and Reynolds, 2004). Regarding 387 sediments, those receiving runoff (SedR) were significantly different to those without receiving runoff (Sed NR) (PERMANOVA runoff: F(1)= 1.5180; p < 0.05), showing the 388 389 influence of the runoff in shaping the functional microbial community structure. In addition,

390 the effect of runoff was different according to presence of plant (PERMANOVA plant: F(1) =391 1.9498; p < 0.01), which was in accordance with previous report showing the influence of 392 plants in the runoff, affecting the amount of water runoff (Nagase and Dunnett, 2012).

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4 3.3. Functional gene indicators

395 **3.3.1. Gene family indicators**

396 In order to obtain predictive association indices (r.g) correlating the abundance of specific 397 functional gene family to conditions, *i.e.* gene family indicators, a multi-level pattern analysis 398 (MLPA) was performed. The MLPA revealed that among the 372 genes families present on 399 the DNA-array, 54 gene families (14.5 %) were significantly associated to a particular 400 condition or other combinations of conditions (Figure 3). Soil and sediment were 401 characterized by gene family indicators belonging to the following gene categories: carbon 402 cycle, organic remediation, nitrogen cycle and metal homeostasis, the first two being 403 predominantly represented (Figure S1). The carbon and nitrogen cycle categories represent 404 main microbial functions involved in organic matter decomposition and nutrient recycling 405 (Burdige, 2011; Levy-Booth et al., 2014; Trivedi et al., 2016). The two other categories 406 represent functions involved in response to the presence of organic and metallic pollutants 407 (Cébron et al., 2008; Michas et al., 2017) substantiating the legacy effect. Sediments 408 receiving runoff (SedR) had more gene family indicators than soils and sediments without 409 runoff (Figure 3). It is likely that sediments exhibit higher diversity of gene family indicators 410 because they are at the mixing land-sea interface area providing a large spectrum of 411 compounds, which require various microbial functions (Groffman and Bohlen, 1999; Guo et al., 2018; Wang et al., 2012). Noteworthy, in sediment receiving runoff (SedR), any gene 412 413 family indicator was identified neither for sediment receiving runoff from planted soil SedR P, 414 neither for sediment with runoff from planted and HC spiked soil (SedR PNC) the HC spiking 415 and in presence of plant. Further indicator analyses were performed at the GVs level among

the gene families related to the HC remediation, focusing in sediments, which represents themixing zone in the land-sea continuum.

418

419 3.3.2. GVs indicators

420 The identification of community coalescence GVs indicators in sediments was carried out on 421 functional genes involved in the HC remediation including 7374 GVs belonging to 32 gene 422 families which were selected based on DNA-array classification, KEGG pathways (Kanehisa 423 and Goto, 2000) and Aromadeg database (Duarte et al., 2014). Among the 7374 GVs, 873 424 GVs, distributed in 7 family subcategories, were significantly associated to either a condition or a combination of conditions (Figure 4 and Figure 5). GVSs indicators were identified for 425 426 the 6 conditions (SedR NPNC, SedR NPC, SedR PNC, SedR PC, SedNR NC, SedNR C) 427 (Figure 4). However, sediments receiving runoff (SedR) exhibited more GVs indicators than 428 those without runoff (SedNR), irrespective of the presence of plants and HC spiking (Figure 4 429 and Figure 5). The GVs indicators belonged to three main function families involved in sulfite 430 reduction (dsrA/B), denitrification (narG, nirK, nirS, and nosZ) and polyaromatic remediation 431 (PAH-ring hydroxylating dioxygenases, PAH-rhd) (Figure 5). It was not surprising to find GVs 432 indicators related to aerobic and anaerobic conditions because oxic/anoxic oscillations were 433 favored in microcosm experiment through tidal cycles. Such oxic/anoxic oscillations have 434 been described in coastal marine sediments (Cravo-Laureau and Duran, 2014), which 435 influence the organization of sulfate reducers (Stauffert et al., 2015), denitrifiers (Stauffert et 436 al., 2014) and HC degraders (Stauffert et al., 2013).

In addition, higher diversity of GVs indicators was observed in sediments receiving runoff
(SedR) than those without (Sed NR) (Figure 5 A). Such observation suggests that runoff
enriched sediments with nutrients and microorganisms via coalescence (Châtillon et al.,
2023) increasing functional diversity. HC spiked sediments receiving or not runoff exhibited
the same categories of GVs indicators (Figure 5 A). Similarly, the pattern of the categories of

442 GVs indicators was similar in sediments receiving runoff from planted soil or non-planted 443 soils (Figure 5 B). However, the differences between these conditions were observed only at 444 the GVs levels (Figure 4 and Figure 5), supporting the functional redundancy. Such 445 functional redundancy has been previously described in HC polluted marine coastal 446 sediment (Cravo-Laureau and Duran, 2014; Stauffert et al., 2013), which corresponds to 447 known mechanisms involved in adaptive response of microorganisms to environmental 448 perturbation (Allison and Martiny, 2008). In conclusion, our results showed that increasing 449 the resolution of the analysis of indicators at the scale of the GVs was relevant to decipher 450 the influence of the conditions in structuring the microbial functional potential. Consistently, 451 similar observations have been reported for taxonomic analysis showing that environmental 452 perturbations are observed only at lower taxonomic level (Kleindienst et al., 2016; Terrisse et 453 al., 2017). The identified GVs indicators play probably a key role in structuring the microbial 454 functional network.

455

456 **3.4.** Functional co-occurrence network

457 In order to determine the keystones GVs, a co-occurrence network analysis was performed. 458 Based on correlation relationship and dissimilarity indices, a functional co-occurrence 459 network was constructed for the conditions with or without runoff, combining GSV indicators 460 from soil and sediment (Figure 6). The network without runoff included 173 nodes and 334 461 significant edges, while the network with runoff included 174 nodes and 445 significant edges 462 (Table 3). The network density, the clustering coefficient, the average number of neighbours 463 and the characteristic path length were higher in the network with runoff (Table 3). These 464 observations suggest that terrigenous inputs resulted in the reorganization of the GSV 465 associations with an increase of the network complexity. The network with runoff showed 466 higher modularity (eight modules representing 25% of the nodes) than the network without 467 runoff (five modules representing 17% of the nodes) further supporting the increase of 468 network complexity due to terrigenous inputs. Such increase in network complexity has been

469 observed in coastal sediment depending on the land-use (Zou et al., 2020) and in PAH polluted sediment (Yan et al., 2021). According to the DNA-array taxonomy, each module in 470 471 both networks included GVs affiliated to diverse bacterial taxa, suggesting that a module 472 represents communities rather than single populations. The major modules (with highest 473 scores and more than three nodes, Table S4) were characterized by the presence of at least 474 one gene involved in the HC degradation and genes involved in the sulfite reduction and 475 denitrification (Figure 6). Although GVs were shared by both networks, they showed different 476 associations, that resulted in different modules, confirming the difference between the 477 networks. Accordingly, the keystone GVs (i.e. highly abundant and connected) identified in 478 the major modules were different in both networks (Table S4 and Table S5) exhibiting similar 479 function. It is likely that the functional redundancy, observed at the GSV level, sustains the 480 main environmental functions despite the reorganization of the networks due to terrigenous 481 inputs.

482

483 **4. Conclusion**

484 This study showed that the removal of HC in sediment was not affected by the soil runoff 485 despite the enrichment in organic matter and microorganisms. However, functional genes 486 were not enriched in sediment receiving runoff but the GV composition was different 487 identifying GV indicators. The terrigenous inputs increased the network complexity and 488 modularity without affecting the overall functions highlighting the functional redundancy. The 489 experimental approach mimicking coalescence zone occurring in the land-sea continuum 490 combined with analyses at the GV level revealed that functional redundancy is the main 491 structural response even in mixing environment. Our results provide helpful functional 492 information for the monitoring and management of coastal environment affected by human 493 land-based activities.

494

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506

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Table 1. Removal percentages of deuterated hydrocarbons spiked in soil and sediment, after 39 days of incubation.

The values are presented as mean \pm SD (n=3).

Compartment		Soil		Sed		
				Sec	JR	SedNR
Condition		NPC	PC	NPC	PC	С
Domoval 0/	Hexadecane	28.8 ± 10.7	29.7 ± 1.9	0.0	0.0	0.0
Removal %	Phenanthrene	95.5 ± 0.6	94.6 ± 1.6	65.4 ± 5.9	75 ± 9.4	64.7 ± 9

Soil: soil; Sed: sediment; SedR: sediment receiving runoff; SedNR: sediment without runoff (control); NP: no plant; P: plant; C: HC spiking.

Table 2. Functional gene variants diversity indices and evenness

	Soil			Sed						
Microarray analysis	NPNC	PNC	NPC	PC	NRNC	NRC	NPNC	PNC	NPC	PC
Coverage (%) ¹	65.1	68.1	65.4	64.6	67.5	68.0	75.3	68.1	79.4	56.1
Richness (R)	37423 ± 695	39173 ± 2103	37616 ± 463	37135 ± 1375	38794 ± 5388	39076 ± 6281	43292 ± 5444	39135 ± 1099	45641 ± 1277	32274 ± 1751
Exponential Shannon (¹ D)	14491 ± 141	14850 ± 1037	13422 ± 1131	14194 ± 510	14634 ± 1657	15412 ± 2211	16210 ± 1383	15214 ± 694	18252 ± 698	12903 ± 1103
Inverse-Simpson (² D)	8581 ± 148	8791 ± 998	6977 ± 1962	8301 ± 652	7740 ± 875	8717 ± 1459	8483 ± 562	8175 ± 605	10778 ± 366	7020 ± 853
Evenness (E)	0.229 ± 0.002	0.224 ± 0.016	0.185 ± 0.050	0.223 ± 0.010	0.200 ± 0.007	0.224 ± 0.018	0.198 ± 0.025	0.209 ± 0.014	0.236 ± 0.003	0.217 ± 0.015

¹ Coverage = ratio of the detected probes / total number of probes available on GeoChip 5S*100

	Without Runoff	With Runoff	
Number of nodes	173	174	
Numbers of edges	334	445	
Positive edge percentage	93.4 %	95.3 %	
Average number of	3.861	5.145	
neighbours			
Characteristic path length	2.163	2.359	
Clustering coefficient	0.117	0.135	
Network density	0.011	0.015	
Connected components	16	9	
Number of modules	5	8	

Table 3. Topological properties of the networks generated for GVs indicators in the conditions without or with runoff.

Figure caption

Figure 1. Schematic representation of the microcosms and the different applied conditions. Soil: soil; Sed: sediment; SedR: sediment receiving runoff; NR: sediment without runoff (control); NP: without plant; P: with plant; NC: without HC spiking; C: withHC spiking.

Figure 2. NMDS based on the Bray Curtis metrics showing the different conditions of soil and sediment compartments overall the gene sequence variants (A) and for the GVS from the organic remediation category (B). Coloured segments link the three replicates from a condition to their average point. Soil: soil; Sed: sediment; SedR: sediment receiving runoff; SedNR: sediment without runoff (control); NP: no plant; P: plant; NC: no deuterated HC spiking; C: deuterated HC spiking.

Figure 3. Functional gene family indicators for the different conditions of soil and sediment compartments. The heatmap represents the z- scores of indicators at the gene family level. The z-scores were computed on the gene family basis by subtracting the mean and dividing by the standard deviation of the florescence intensity (blue for the low fluorescence intensity and orange for the high fluorescence intensity). On the left of the heatmap, the row annotations indicate the condition (i) and the gene family category (ii). The data represent the mean of three biological replicates (n=3). Soil: soil; Sed: sediment; SedR: sediment receiving runoff; SedNR: Sed without runoff (control); NP: no plant; P: plant; NC, no deuterated HC spiking; C, deuterated HC spiking.

Figure 4. Functional HC biodegradation gene indicators for the different sediment conditions. The heatmap represents the z- scores of bioindicators at the GVs level. The z-scores were computed on the GVs basis by subtracting the mean and dividing by the standard deviation of the florescence intensity (blue for the low fluorescence intensity and yellow for the high fluorescence intensity). On the left of the heatmap, the row annotations indicate the condition (i) and the gene family subcategory from the organic remediation category (ii). The data represent the mean of three biological replicates (n=3). Sed: sediment; SedR: sediment receiving runoff; SedNR: Sed without runoff (control); NP: no plant; P: plant; NC: no deuterated HC spiking; C: deuterated HC spiking.

Figure 5. Distribution of the different GSV subcategories of organic remediation cateory in sediment (A) with (SedR) or without (SedNR) runoff, (B) with (Sed C) or without (Sed NC) HC spiking, (C) planted (SedR P) or non-planted (SedR NP) soil with runoff. The number of GVs for each subcategory is indicated in bracket.

Figure 6. Functional co-occurrence networks of soil and sediment receiving runoff (SedR; A) or without runoff (SedNR; B). The nodes were coloured according to the different modules (grey is used for unclustered nodes). The edges represent a robust (convergence of the measures) and significant association (p < 0.05) between nodes. The size of each node is proportional to their weight and the thickness of the edges is proportional to each association coefficient between GVs. On the right, modules of co-occurring microbial GVs of soil and sediment receiving runoff (SedR; A) and in soil and sediment without runoff (SedNR; B) at the end of the experiment based on correlations and dissimilarities analysis. Within modules, the border of the nodes matched the gene categories.





0
0



Se	dR	Se

NPC

PC

NC



(i) Condition

Soil Soil PNC Soil NPC Soil PC

Sed Sed NC Sed C

SedR SedR NC SedR C SedR NP SedR NPNC SedR NPC SedR NPC SedR NPC

SedNR SedNR NC SedNR C

Soil&SedR Soil&SedR P Soil&SedR PNC

Soil&SedNR Soil&SedNR NC

(ii) Gene category







(ii) Gene subcategory

- Aromatics
- Carbon degradation
- Denitrification
- Herbicides related compound
- Other Hydrocarbons
- Polycyclic aromatics
- Sulfite reduction

B

A

With HC spiking (Sed C)

Without HC spiking (Sed NC)

Planted soil with runoff (SedR P)

Sulfite reduction

Non-planted soil with runoff (SedR NP)

Denitrification (17)

C

(21) Oth Her Carl Aromatics (16)

Other hydrocarbons (1)
 Herbicids related compounds (1)
 Carbon degradation (1)

Sulfur cycling

Organic remediation

△ Sed