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1 **Meta-omics provides insights into the impact of hydrocarbon contamination on**
2 **microbial mats functioning**

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23

24

25 **Abstract**

26 Photosynthetic microbial mats are stable, self-supported communities. Due to their
27 coastal localization, these mats are frequently exposed to hydrocarbon contamination and are
28 able to grow on it. To decipher how this contamination disturbs the functioning of microbial
29 mats, we compared two mats: a contaminated mat exposed to chronic petroleum
30 contamination and a reference mat. The taxonomic and metabolic structures of the mats in
31 spring and fall were determined using metagenomic and metatranscriptomic approaches.
32 Extremely high contamination disturbed the seasonal variations of the mat. ABC transporters,
33 two-component systems and type IV secretion systems related genes were overabundant in
34 the contaminated mats. Xenobiotic degradation metabolism was minor in the metagenomes of
35 both mats and only the expression of genes involved in Polycyclic Aromatic hydrocarbon
36 degradation was higher in the contaminated mat. Interestingly, the expression rates of genes
37 involved in hydrocarbon activation decreased during the 1-year study period, concomitant
38 with the decrease in easily degradable hydrocarbons, suggesting a transient effect of
39 hydrocarbon contamination. *Alteromonadales* and *Oceanospirillales* hydrocarbonoclastic
40 bacteria appeared to be key in hydrocarbon remediation in the contaminated mat. Overall, the
41 contaminated microbial mat was able to cope with hydrocarbon contamination and displayed
42 an adaptive functioning that modified seasonal behaviour.

43

44 **Introduction**

45 Photosynthetic microbial mats growing in coastal areas are among the most diverse and
46 complex marine ecosystems [1]. The overall metabolism of a microbial mat is driven by solar
47 light as the main energy source. Microbial activities generate physical and chemical gradients
48 in the mat that maintain the structure and activity of the mat's community [2], leading to a
49 stable, self-supported mat. Microbial mats are highly dynamic and are subjected to strong
50 fluctuations at the diel or seasonal scales [2]. The extensive genetic and metabolic diversity of
51 photosynthetic microbial mat-inhabiting organisms enable development in a wide variety of
52 environments, including hot springs, hypersaline and alkaline environments and polar ponds
53 [1, 3–6]. Due to their localization in coastal zones, microbial mats are frequently subjected to
54 hydrocarbon contamination [7, 8]. The response of microbial mats to petroleum
55 contamination has been studied in microcosm experiments and under natural conditions after
56 acute pollution events (accidental or laboratory experiments). Microbial mats can cope with
57 contamination and develop a robust community adapted to petroleum levels [8, 9]. The few
58 studies that have focused on chronic oil pollution have concluded that the structure of
59 microbial mats is related to the level of contamination [8] and that contaminated mats can
60 degrade hydrocarbons [10, 12]. Generally, noticeable changes in composition [8, 10] of high
61 contaminated mats are reported, and some studies described lower diversity in higher
62 contaminated mats [13], but not overabundance of hydrocarbonoclastic microorganisms have
63 been observed. Nevertheless, when these contaminated mats were submitted to fresh oil
64 pollutions, an immediate induction of RHD genes involved in polyaromatic hydrocarbon
65 degradation was observed, accompanied with an efficient degradation of crude oil [10]. This
66 metabolic response (associated with a community structural shift [12]) was however quickly
67 reversed, highlighting a fast, adaptive and efficient response of the metabolically active
68 bacterial population. Oil contamination also impacts typical seasonal behaviour of

69 photosynthetic microbial mats, by strongly inhibiting primary production and respiration in
70 spring, but with no significant impact in fall [20]. As consequences, the typical
71 overabundance of *Cyanobacteria* in fall compared to spring could not be observed in the
72 higher contaminated mat [19]. These studies were performed using taxonomic and/or
73 metabolic gene markers, such as ribosomal genes or genes involved in hydrocarbon
74 degradation [9–14].

75 High-throughput sequencing of metagenomes and metatranscriptomes has provided an
76 unprecedented overview of the functional capacities and gene expression of microbial
77 communities confronting environmental stresses. As the presence and expression of genes
78 involved in hydrocarbon degradation (including complete metabolic pathways) have been
79 observed in oil spill-contaminated seawaters [15–17] and soils [18, 19], applying these
80 sequencing techniques to contaminated microbial mats will provide new insights on the
81 adaptation of microbial mats to hydrocarbon contamination.

82 The aim of this paper was to elucidate the impact of long-term petroleum
83 contamination on the functioning of microbial mats. Two photosynthetic microbial mats
84 located in the Berre lagoon, a brackish lagoon bordering the Mediterranean Sea in the South
85 of France, were investigated in this work. These mats feature similar physical and chemical
86 parameters (salinity, temperature, solar irradiation, etc.) [20] but have been subjected to
87 differing hydrocarbon contamination levels. Specifically, the contaminated mat received
88 hydrocarbon inputs from a petrochemical industry site for more than 80 years. By applying
89 metagenome and enriched mRNA metatranscriptome sequencing, we described the key
90 metabolic pathways of both microbial mats. Comparing the contaminated mat with the
91 reference mat revealed the role of petroleum pollution in microbial community functioning. A
92 deeper analysis of metabolism related to hydrocarbon-degradation pathways was also
93 performed.

94 **Material and methods**

95 **Sampling sites and procedure**

96 Berre lagoon is located on the Mediterranean French coast. Microbial mat samples
97 were collected from two sites in the lagoon: a reference station located in the bird reserve
98 “Les salins du Lion” (SL) (43.452570 N 5.230085 E) and a contaminated station located in a
99 retention basin receiving hydrocarbon-contaminated rainfall water from a petrochemical
100 industry site (EDB1) (43.484946 N 5.188452 E) [8, 21]. At this latter site, the hydrocarbon
101 content reaches 96 µg/g [8]. In addition to this chronic contamination, an accidental oil spill
102 occurred in September 2009 due to the overflow of a hydrocarbon-polluted water retention
103 pond [22], which increased the hydrocarbon content. Three sampling exercises were
104 performed: one in spring (April 2012) and two in fall (September 2011 and 2012), which were
105 named Apr12, Sept11 and Sept12, respectively. A piece of mats (around 1.5 m²), including
106 the top two centimetres of sediment were collected at mid-day as described in [19].
107 Subsamples for molecular analyses were stored at -80°C. A description of main physical-
108 chemical characteristics and hydrocarbon composition of both sites can be found at Table S1.

109 **Whole meta-genome and meta-transcriptome sequencing and bioinformatics procedures**

110 DNA and RNA were co-extracted in triplicate using an RNA PowerSoil Total RNA
111 Isolation Kit (MO BIO Laboratories, Inc.) coupled with an AllPrep DNA/RNA Mini Kit
112 (QIAGEN) for DNA and RNA separation. RNA was digested with an RNase-Free DNase Set
113 (QIAGEN) for DNA removal, and RNA quality was checked as described by Aubé and
114 coworkers [20]. Genomic DNA libraries were constructed using a NEXTFlex PCR-free DNA
115 Sequencing Kit from BioScientific PCR-free kit following the manufacturer's protocol. RNA-
116 seq libraries were prepared according to Illumina's protocol on a Tecan EVO200 liquid
117 handler. An Illumina TruSeq Stranded mRNA sample prep kit was used to analyse RNA after

118 mRNA enrichment using an Epicentre Ribo-Zero rRNA removal kit. RNA was fragmented to
119 generate double-stranded cDNA. Ten cycles of PCR were used to amplify libraries following
120 manufacturer's instructions (Illumina TruSeq Stranded mRNA library prep kit, Cat. No. RS-
121 122-2101), and the libraries were precisely quantified by qPCR using a KAPA Library
122 Quantification Kit. RNA-seq and gDNA sequencing were performed with an Illumina
123 HiSeq2500 instrument using a paired-end read length of 2×100 bp with an Illumina TruSeq
124 SBS sequencing kit v3.

125 The Trim Galore! utility was used for read quality control
126 http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). Possible adapter sequences
127 based on the Illumina TruSeq Adapter index were removed from the reads. Data filtering was
128 performed with cutadapt [23] and FASTX-Toolkit
129 (http://hannonlab.cshl.edu/fastx_toolkit/index.html) by trimming reads with a Q score of less
130 than 15 at the 3' end, trimming unidentified dNTP (N) at the read ends and discarding reads
131 with Ns>10%. Reads shorter than 50 bp with low-quality bases (Q<20) were also discarded.
132 For functional and taxonomic annotations, all filtered reads (DNA and mRNA) were aligned
133 to the KEGG gene database [24] using LAST aligner v392 [25]. Each read was then annotated
134 using the single-directional best hit information method [26] with an identity cut-off of 60%
135 and a score of 96. Eukaryotic sequences were removed from the dataset. Statistics of the reads
136 at each bioinformatics analysis step are shown in the supplementary material (Table S2).

137 **Statistical analysis**

138 Before statistical analysis, random sampling of filtered data was performed to obtain
139 the same number of reads per sample (i.e. the minimum number of sequences in metagenomic
140 or metatranscriptomic samples (Table S2)) and the means of the pseudo-triplicates were
141 calculated to perform statistical tests. Taxonomic biomarkers of hydrocarbon contamination
142 were detected using the LefSe algorithm [27]. The first analysis step was a non-parametric

143 Kruskal-Wallis (KW) sum-rank test to detect taxa and functional pathways with significant
144 differential abundances. Biological consistency was subsequently investigated using a
145 pairwise Wilcoxon test. Finally, linear discriminant analysis (LDA) was used to estimate the
146 effect size of each differentially abundant taxon. Alpha values of 0.05 were used for the KW
147 and Wilcoxon tests, and a threshold of 2 was used for the logarithmic LDA scores. Gene and
148 transcript abundances for functional data were compared using a two-group White's non-
149 parametric t-test in STAMP [28] by KEGG subsystem. To account for the variation of
150 transcriptional activity, the gene expression rate was calculated as described by Stewart et al
151 [29]. Hydrocarbon content were analysed by principal component analysis (PCA) using the
152 FactoMineR package [30]; variables with cos2 below 0.5 on each factorial plane are not
153 shown on the corresponding correlation circles of the PCA.

154 **Accession number**

155 The sequence data are available in the Sequence Read Archive of the National Center
156 for Biotechnology Information under accession number SRP063590.

157

158 **Results and discussion**

159 Microbial mats are highly stratified systems, in which microorganisms' metabolism
160 supports their physical and chemical structure. In return, physical and chemical conditions of
161 the mats favours microbial specific metabolisms: structure and function are thus slightly
162 dependent. The goal of the present study was to determine if pollution changes metabolic
163 profiles and its consequences on coastal mat's structure and functioning.

164 **Structure of microbial mat communities**

165 The metagenomes and metatranscriptomes of the microbial mats were annotated using
166 the KEGG database. Between 24.70% and 46.86% of the metagenome and metatranscriptome
167 sequences, respectively, aligned to the KEGG database (Table S2). The community
168 composition based on the taxonomic affiliations of protein-encoding genes revealed a
169 structure similar to that described by 16S rRNA gene affiliations [20] and to those of other
170 mats [31, 32]; i.e. *Proteobacteria*, *Cyanobacteria*, and *Bacteroidetes* were dominant (Fig. 1)
171 with metagenomes mean relative abundance of 48.1%, 16.0% and 15.5% respectively. The
172 high similarity between the structure described here and those described in previous studies
173 on the same mats based on 16S rDNA sequences [8, 19], supported the robustness of the
174 annotation approach. *Oscillatoriales* dominated among *Cyanobacteria*. The relative
175 abundance of *Cyanobacteria* was highest in the EDB1 Sept12 metagenomes and
176 metatranscriptomes, with a greater abundance of *Rivulariaceae* (Table S3). Among the
177 *Deltaproteobacteria* (10.8% of the metagenomes mean relative abundance) most of the
178 sequences were affiliated with sulphate reducers in the order *Desulfobacterales* (Table S3).
179 The sequences related to *Gammaproteobacteria* (15.0% of the metagenomes mean relative
180 abundance) were affiliated with *Alteromonadales* and *Chromatiales*; most of the
181 *Chromatiales* were purple sulphur bacteria. Among the *Alpha* and *Betaproteobacteria* (17.1%

182 and 4.3% of the metagenomes mean relative abundance), *Rhodobacterales* and
183 *Burkholderiales* dominated, respectively. Consistent with observations in the Guerrero Negro
184 hypersaline microbial mats and Highborne Cay mat (The Bahamas) [33, 34], *Archaea* were in
185 the minority in the microbial communities, accounting for 1% to 3% of total reads and mainly
186 represented by the methanogens *Methanomicrobia*. Thus, both mats are classical mats with
187 dominance of photosynthetic bacteria and bacteria involved in the sulphur cycle (sulphate
188 reducers and sulphur oxidizers [2]).

189 The community structures described using metatranscriptomic data differed from
190 those described by metagenomic data (Fig. 1). Remarkably, transcripts related to
191 *Cyanobacteria* highly dominated the communities in both mats, accounting for 36.5% to 78%
192 of the reads, highlighting the major role of *Cyanobacteria* compared to other functional
193 groups in the mats [34, 35].

194 As microbial mat functioning is dependent on the seasonal period [36], sampling was
195 performed in spring and fall to assess the seasonal variation of the structure and activity of the
196 mats. The reference mat showed typical seasonal variations characterized by 1) an increase of
197 *Cyanobacteria* in fall (around 13% of metagenomic data in spring and 19% in fall, Table S3);
198 and, 2) an increase of the sulphur related microorganisms in spring. This increase was
199 observed either at metagenomic (from near 14% in fall to 19% in spring) or at
200 metatranscriptomic level (a mean of 6% in fall and 21% in spring, Table S3) and mainly
201 concerned *Desulfobacterales* and *Chromatiales*. On the contrary, no seasonal variations were
202 observed in the contaminated mat: *Cyanobacteria* highly dominated the community in Sept12
203 but not in Sept11 samples, and a continuous decrease of the relative abundance of
204 microorganisms related to sulphur cycle, either at metagenomics or metatranscriptomic level,
205 was observed in the contaminated mat (Table S3).

206 **Metabolism of microbial mat communities**

207 A total of 7,009 different KEGG ortholog groups (KOs) were detected in the
208 metagenome, and 6,470 KOs were detected in the metatranscriptome. Among the annotated
209 reads, around 44% of the microbial mat metagenomes matched genes involved in pathways
210 associated with metabolism (Fig. 2); including 10.2–11.1% related to genes involved in
211 carbohydrate metabolism (547 KOs); and 9.9–10.8% related to amino acid metabolism (420
212 KOs). Energy metabolism-related sequences represented only 4.1–4.5% of the metagenomes
213 but up to 49.9% of the metatranscriptomes (389 and 381 KOs for genes and transcripts,
214 respectively). Among them, photosynthesis metabolism-related genes (i.e. genes encoding
215 proteins of photosystems I and II (P700 and P680) and phycobiliproteins (phycocyanin,
216 allophycocyanin and phycoerythrin subunits)) exhibited maximum expression rates of 1094.
217 Genes related to nitrogen and sulphur metabolism exhibited high expression rates as well.
218 Sequences related to nitrogen metabolism were mainly involved in nitrogen fixation (i.e.
219 nitrogenase enzyme complex); among them, *nifH* exhibited expression rates as high as 5.85.
220 The sequences related to the sulphur cycle were involved in sulphate reduction: adenylyl-
221 sulphate reductase and sulphite reductase (AprAB and DsrAB) exhibited maximum
222 expression rates of 8.83 (data not shown).

223 While reference mat showed a typical seasonal behaviour, contaminated mat did not
224 show the same behaviour. In this case, the comparison of metabolic potentials of both mats in
225 order to define which of them are related to the contamination is challenging. For further
226 analysis on hydrocarbon contamination impact on microbial mat functioning, all the samples
227 for each mat (reference or contaminated) have been averaged. This approach could probably
228 dissimulate some metabolisms, but eliminates the errors originating from a seasonal divergent
229 functioning.

230

231 **Metabolic and taxonomic differences between sites with focus on metabolic pathways**
232 **associated with xenobiotic degradation**

233 Microbial communities from coastal environments impacted by punctual oil input
234 have been intensively studied (e.g. [16, 37, 38]), and a decrease in microbial diversity
235 associated with dominance of hydrocarbon-degrading marine bacteria is generally observed
236 following an oil spill [39]. By contrast, in this study, dominance of hydrocarbonoclastic
237 bacteria in the contaminated mat compared to the reference mat was not observed, consistent
238 with previous studies of chronically contaminated sites [8, 40]. At the taxonomic level, 36 and
239 3 differentially abundant taxa were detected based on metagenomic and metatranscriptomic
240 data, respectively (Fig. 3). At the metagenomic level, the main difference was a decrease in
241 the relative abundance of some phylogenetic groups (Fig. 3a), suggesting a sensitivity of those
242 groups to contamination. At the transcriptomic level (Fig. 3b), few taxa appear characteristic
243 of each mat. Only *Parvularculales* within *Alphaproteobacteria*, were characteristic taxa of
244 the contaminated mat. *Alphaproteobacteria* are key contributors to the later stages of oil
245 degradation [41]. However, hydrocarbon degradation is strain specific; phylogenetic
246 description based on taxonomic genes such as 16SrRNA (especially when this description is
247 performed at high phylogenetic levels such as the order) has low explanatory value, since two
248 strains belonging to the same taxon can be or not hydrocarbonoclastic. Using short reads
249 approaches, affiliation of sequences below the genus, or even the family is often unattainable.

250 The greatest differences in metabolic potential at the metagenomics level were related
251 to signal transduction and membrane transport. These pathways were significantly more
252 abundant in the hydrocarbon-contaminated microbial mat, whereas nucleotide metabolism
253 and replication and repair pathways were significantly more abundant in the reference mat

254 (Fig. 4). The contaminated mat was enriched with genes involved in two component systems
255 (signal transduction, TCS) of the OmpR family (involved in copper ion efflux and manganese
256 transport as a response of Mn starvation) and NtrC family (involved in nitrogen availability)
257 (Table S4). The TCS are known to modify microbial physiology in response to multiple
258 environmental signals and thus playing a role in biogeochemical cycles. Indeed, marine
259 bacteria have varied TCS systems, and it has been proposed that lacks of TCS could be a
260 hallmark of oligotrophy in marine systems [42]. Contaminated mat is probably enriched with
261 TCS as an adaptive response to contamination. The overabundance of membrane transport
262 related genes concern the ABC transporters and the type IV (T4SS) secretion systems.
263 Recently, Xu et al. [43] described an enrichment in ABC transporters and TCS after dibutyl
264 phthalate contamination in soils. They also observed an acceleration of nitrogen, carbon and
265 sulfate metabolisms and suggested that ABC transporters and TCS were the culprits of this
266 metabolic activation.

267 The T4SS are found in Gram negative bacteria, and are capable of secrete a wide
268 variety of substances across the bacterial membranes including toxic bacterial effectors that
269 result in cell death of rival bacteria and eukaryote [44]. T4SS is ancestrally related to bacterial
270 DNA conjugation systems, and the T4SS-mediated genetic horizontal transfer is considered
271 as a major contributor to bacterial genomic mobility [45]. Altogether, enrichment of ABC
272 transporters and T4SS appears as a mechanism to control the community structure and the
273 metabolic potential in the contaminated microbial mat.

274 Although less pronounced, xenobiotic biodegradation and metabolism pathways were
275 significantly more abundant in the contaminated mat than in the reference mat. At the
276 metatranscriptomic level, no differences in xenobiotic biodegradation and metabolism nor in
277 the others level1 KEGG metabolic pathways were observed between the two mats (p-
278 value>0.05 or difference in mean proportions <0.05%).

279 To further characterize the hydrocarbon degradation potential of the mats, genes
280 involved in hydrocarbon molecule activation were retrieved from the whole dataset (Fig. 5a,
281 Table S5). These genes are specifically associated with hydrocarbon degradation, whereas
282 those involved in intermediate reactions can be shared with other metabolic pathways
283 (<http://www.genome.jp/kegg/pathway.html>). The gene encoding alkane 1-monooxygenase
284 (*alkB*) (the single representative of genes involved in the fatty acid degradation pathway,
285 Table S5), which is responsible for alkane activation, was more abundant (Wilcoxon test, p-
286 value = 0.03125) than the genes involved in degradation of aromatic compounds (Fig. 5a), as
287 observed after the Deepwater Horizon oil spill [16]. The relative abundance and taxonomic
288 identification of *alkB* were similar between the contaminated and reference mats (Fig. 5a and
289 S1). Although the *alkB* gene has been widely used to investigate alkane degradation [46–49]
290 and as a marker of petroleum degradation potential [16], its direct relationship with petroleum
291 hydrocarbon degradation is questionable, especially in chronically contaminated
292 environments [14]. In fact, *alkB* is involved in the degradation of alkanes of both biogenic and
293 petrogenic origin. Biogenic alkanes are the consequence of organic matter degradation and are
294 ubiquitous in nature [50]. As for *alkB*, the relative abundance of all the genes involved in the
295 degradation of aromatic hydrocarbons was similar in both mats (Fig. 5a).

296 Analysis at the metatranscriptomic level revealed significant higher expression of
297 genes involved in PAH activation in the contaminated mat (Fig. 5b). However, the expression
298 of all the genes involved in HC activation in the contaminated mats decreased over time (Fig.
299 6a). Interestingly, this decrease was concomitant with changes in hydrocarbon composition
300 during the sampling exercises (Fig. 6b). Short-chain alkanes (<C₂₈), medium-weight PAHs,
301 and heavy PAHs dominated the hydrocarbons in the first, second, and last exercises,
302 respectively. The contaminated mat was therefore enriched in recalcitrant molecules in the
303 last exercise [20], reaching a composition similar to that previously observed at this site [8].

304 Overall, these results (chemical modification and a decrease in the expression rates of
305 hydrocarbon activation-involved genes) suggest a progressive degradation of the more easily
306 degradable hydrocarbons released by the overflow spill in September 2009.

307 A decrease in relative abundances was evident for fatty acid degradation activation
308 genes, namely *alkB* (Fig. 7a). The taxonomic affiliations of the *alkB* transcripts suggest
309 *Alteromonadales*-related bacteria as the main alkane degraders in the first sampling exercise,
310 consistent with a previous study of the Berre lagoon in which *Marinobacter aquaeolei* was
311 the dominant *alkB* phylotype [14]. The relative abundance of *alkB* transcripts decreased over
312 time in parallel with the decrease in lower-molecular-weight alkanes. Transcripts involved in
313 PAH degradation also decreased, albeit to a lesser extent, with *Oceanospirillales*-related
314 bacteria as the main actors (Fig. 7b).

315 **Conclusion**

316 Both mats displayed a typical structure and functioning, with dominance of
317 *Cyanobacteria* and sulphur cycle related microorganisms.. Genes involved in environmental
318 information processing were overabundant in the contaminated mat compared to the reference
319 one. However, xenobiotic degradation metabolisms represented a minor part of the
320 metagenome and metatranscriptome, and no overabundance of hydrocarbonoclastic bacteria
321 was observed in the contaminated mat. The expression levels of genes associated with
322 hydrocarbon degradation pathways varied between the mats and among sampling exercises.
323 In the contaminated mat, the expression of genes responsible for hydrocarbon activation was
324 related to hydrocarbon composition, suggesting degradation of easily degradable molecules.
325 The studied mat appeared robust enough to cope with hydrocarbon contamination but its
326 seasonal behaviour was affected.

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331

332

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481

482 **Figure captions:**

483

484 **Fig. 1** Metagenome (DNA based) and metatranscriptome (mRNA based) taxonomic structure
485 at the class level based on the affiliation of sequences. The figure shows the fraction of reads
486 that hits with the KEGG database. Data represent the mean of triplicates. EDB1: highly
487 contaminated mat; SL: reference mat. The dashed lines separate from the bottom to the top:
488 *Cyanobacteria*, *Proteobacteria* and *Bacteroidetes*

489 **Fig. 2** Metabolic structure of the metagenome and metatranscriptome of both microbial mats
490 based on KO assignation and pathways of sequences (relative abundance). Data represent the
491 mean of triplicates. EDB1: highly contaminated mat; SL: reference mat. The dashed lines
492 separate the energy metabolic pathways.

493 **Fig. 3** LEfSe analysis of the microorganisms differentially abundant (metagenomes; **a**) and active
494 (metatranscriptomes; **b**) in contaminated (EDB1, red) and reference (SL, green) microbial mats.
495 Roots of cladograms stand for the domain, and concentric circles represent the following
496 taxonomic levels until the order. Only class with p-value below 0.05 are shown.

497

498 **Fig. 4** Comparison (White's non-parametric t-test in STAMP) of functional gene annotations of
499 metagenomes using KEGG pathways (level 1). Plots compare the hydrocarbon contaminated
500 mat (EDB1) in black to the reference (SL) in white. Only pathways with p-value below 0.05,
501 with difference in mean proportions above 0.05% are shown.

502 **Fig. 5** Extended error plots comparison of the hydrocarbon degradation-related genes or
503 transcripts in the metagenome (**a**) and metatranscriptome (**b**) data. Only the genes involved in
504 the activation of the hydrocarbon molecules were considered (Table S5). The plot compares the
505 hydrocarbon contaminated mat (EDB1) in black to the reference (SL) in white.

506

507 **Fig. 6** Expression level of genes involved in hydrocarbon degradation (Table S5) in mats (**a**);
508 in black, contaminated site (EDB1) in white, reference (SL) mat. Principal Component biplot
509 (PCA) based on hydrocarbon contents in EDB1 (**b**): A, Anthracene; ANA, Acenaphtene; ANY,
510 Acenaphtylene; BA, Benzo(a)anthracene; BAP, Benzo(a)pyrene; DBA,
511 Dibenz(a,h)anthracene; F, Fluorene; FL, Fluoranthene; IN, Indeno(1,2,3-cd)pyrene; P,
512 Phenanthrene; PY, Pyrene. C11-C16, sum of alkanes from C11 to C16; C17-C22, sum of
513 alkanes from C17 to C23; C24-C28, sum of alkanes from C24 to C28. The parameters with a
514 \cos^2 below 0.5 are not shown (Benzo(g,h,i)perylene; Chrysene; Naphtalene;
515 Benzo(b+k)fluoranthene).

516
517 **Fig. 7** Relative abundance (%) and affiliation of transcripts involved in the activation of fatty
518 acids (*alkB* transcripts in our study, exclusively) (**a**) and polycyclic aromatic hydrocarbon (**b**)
519 degradation pathways. Figure shows the fraction of reads that hits with the KEGG database.
520 Data represent the mean of the triplicates.