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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 m<sup>2</sup>), 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/](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](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 (<C<sub>28</sub>), 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.