Meta-omics Provides Insights into the Impact of Hydrocarbon Contamination on Microbial Mat Functioning

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HAL Id: hal-03065790
https://hal.archives-ouvertes.fr/hal-03065790

Submitted on 14 Dec 2020

1 **Meta-omics provides insights into the impact of hydrocarbon contamination on**

2 **microbial mats functioning**

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14 Keywords: microbial mats, metabolism, metagenomics, metatranscriptomic, functioning,

15 diversity

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24

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 Polyciclic 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

Introduction

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

Metabolism of microbial mat communities

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

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

230

Metabolic and taxonomic differences between sites with focus on metabolic pathways

associated with xenobiotic degradation

Microbial communities from coastal environments impacted by punctual oil input

have been intensively studied (e.g. [16, 37, 38]), and a decrease in microbial diversity

associated with dominance of hydrocarbon-degrading marine bacteria is generally observed

following an oil spill [39]. By contrast, in this study, dominance of hydrocarbonoclastic

bacteria in the contaminated mat compared to the reference mat was not observed, consistent

with previous studies of chronically contaminated sites [8, 40]. At the taxonomic level, 36 and

3 differentially abundant taxa were detected based on metagenomic and metatranscriptomic

data, respectively (Fig. 3). At the metagenomic level, the main difference was a decrease in

the relative abundance of some phylogenetic groups (Fig. 3a), suggesting a sensitivity of those

groups to contamination. At the transcriptomic level (Fig. 3b), few taxa appear characteristic

of each mat. Only *Parvularculales* within *Alphaproteobacteria,* were characteristic taxa of

the contaminated mat. *Alphaproteobacteria* are key contributors to the later stages of oil

degradation [41]. However, hydrocarbon degradation is strain specific; phylogenetic

description based on taxonomic genes such as 16SrRNA (especially when this description is

performed at high phylogenetic levels such as the order) has low explanatory value, since two

strains belonging to the same taxon can be or not hydrocarbonoclastic. Using short reads

approaches, affiliation of sequences below the genus, or even the family is often unattainable.

The greatest differences in metabolic potential at the metagenomics level were related

to signal transduction and membrane transport. These pathways were significantly more

abundant in the hydrocarbon-contaminated microbial mat, whereas nucleotide metabolism

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 ($<C_{28}$), 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.

327 **Acknowledgement**

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Figure captions:

Fig. 1 Metagenome (DNA based) and metatranscriptome (mRNA based) taxonomic structure

at the class level based on the affiliation of sequences. The figure shows the fraction of reads

that hits with the KEGG database. Data represent the mean of triplicates. EDB1: highly

contaminated mat; SL: reference mat. The dashed lines separate from the bottom to the top:

Cyanobacteria, *Proteobacteria* and *Bacteroidetes*

Fig. 2 Metabolic structure of the metagenome and metatranscriptome of both microbial mats

based on KO assignation and pathways of sequences (relative abundance). Data represent the

mean of triplicates. EDB1: highly contaminated mat; SL: reference mat. The dashed lines

separate the energy metabolic pathways.

Fig. 3 LEfSe analysis of the microorganisms differentially abundant (metagenomes; **a**) and active

(metatranscriptomes; **b**) in contaminated (EDB1, red) and reference (SL, green) microbial mats.

Roots of cladograms stand for the domain, and concentric circles represent the following

taxonomic levels until the order. Only class with p-value below 0.05 are shown.

Fig. 4 Comparison (White's non-parametric t-test in STAMP) of functional gene annotations of

metagenomes using KEGG pathways (level 1). Plots compare the hydrocarbon contaminated

mat (EDB1) in black to the reference (SL) in white. Only pathways with p-value below 0.05,

with difference in mean proportions above 0.05% are shown.

Fig. 5 Extended error plots comparison of the hydrocarbon degradation-related genes or

transcripts in the metagenome (**a**) and metatranscriptome (**b**) data. Only the genes involved in

the activation of the hydrocarbon molecules were considered (Table S5). The plot compares the

hydrocarbon contaminated mat (EDB1) in black to the reference (SL) in white.

Fig. 6 Expression level of genes involved in hydrocarbon degradation (Table S5) in mats (**a**); in black, contaminated site (EDB1) in white, reference (SL) mat. Principal Component biplot (PCA) based on hydrocarbon contents in EDB1 (**b**): A, Anthracene; ANA, Acenaphtene; ANY, Acenaphtylene; BA, Benzo(a)anthracene; BAP, Benzo(a)pyrene; DBA, Dibenz(a,h)anthracene; F, Fluorene; FL, Fluoranthene; IN, Indeno(1,2,3-cd)pyrene; P, Phenanthrene; PY, Pyrene. C11-C16, sum of alkanes from C11 to C16; C17-C22, sum of alkanes from C17 to C23; C24-C28, sum of alkanes from C24 to C28. The parameters with a cos2 below 0.5 are not shown (Benzo(g,h,i)perylene; Chrysene; Naphtalene; Benzo(b+k)fluoranthene).

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