

1 **Aerobic degradation of crude oil by microorganisms in soils**
2 **from four geographic regions of China**

3 Qinglong Liu¹, Jingchun Tang^{*1,2,3}, Kai Gao⁴, Ranjit Gurav¹, John P Giesy^{5,6,7,8,9}

4 ¹College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China

5 ²Tianjin Engineering Center of Environmental Diagnosis and Contamination Remediation, Tianjin
6 300071, China

7 ³Key Laboratory of Pollution Processes and Environmental Criteria (Ministry of Education),
8 Tianjin 300071, China

9 ⁴Tianjin Academy of Environmental Sciences, Tianjin 300191, China

10 ⁵Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

11 ⁶Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon,
12 Saskatchewan, Canada

13 ⁷School of Biological Sciences, University of Hong Kong, Hong Kong, SAR, China

14 ⁸State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment,
15 Nanjing University, Nanjing, People's Republic of China

16 ⁹Department of Biology, Hong Kong Baptist University, Hong Kong, SAR, China

17 * Corresponding author: Jingchun Tang

18 Tel.: +86-22-83614117, Fax: +86-22-83614117

19 E-mail address: tangjch@nankai.edu.cn

20

21

22

23

24 **Table S1.** Summary of sizes of libraries of sequences for operational taxonomic units (OTUs), and
25 diversity indices based on 16S rRNA gene sequences during incubations of soils in microcosms.

Samples	Number of sequences	Number of OTUs	Ace	Chao I richness	Shannon(H')	Simpson	Coverage (%)
DG0d	5777	247	262	263	3.58	0.0347	99.48
DG112d	7872	195	239	238	3.36	0.0723	99.39
SS0d	10029	187	236	240	1.72	0.4767	99.46
SS112d	11802	114	140	135	2.43	0.1643	99.76
NE0d	12297	270	302	312	4.16	0.0643	99.61
NE112d	10673	94	120	121	2.88	0.1904	99.76
XM0d	11238	123	140	138	2.64	0.1562	99.78
XM112d	13854	139	150	152	3.05	0.0874	99.86

26 Calculations based on the operational taxonomic units (OTUs) determined at an evolutionary distance
27 of 0.03.

28

29

30

31

32

33

34

35 **Table S2.** Results of sequencing of DGGE bands.

Sequence	Homologous strains	Identity	accession number in ENA
D0-1	<i>Marinobacter</i>	99%	LN649243
D0-2	Uncultured <i>alpha</i> <i>proteobacterium</i> sp.	100%	LN649242
D0-3	Uncultured <i>Rhodanobacter</i> sp.	98%	LN649253
D8-1	Uncultured <i>Rhodanobacter</i> sp.	99%	LN649253
D16-1	<i>Marinobacter</i>	99%	LN681343
D16-2	<i>Brevundimonas</i> sp.	100%	LN681344
S0-1	<i>Pseudomonas</i> sp.	99%	LN649240
S1-1	<i>Streptomyces</i> sp.	100%	LN649246
S1-2	Uncultured bacterium	98%	LN649248
S16-1	Uncultured <i>alpha</i> <i>proteobacterium</i> sp.	98%	LN681346
N0-1	<i>Pseudomonas</i> sp.	99%	LN649240
N4-1	<i>Burkholderia</i> sp.	100%	LN649249
N8-1	Uncultured <i>alpha</i> <i>proteobacterium</i> sp.	100%	LN649252
N8-2	Uncultured <i>Rhodanobacter</i> sp.	99%	LN649253
X0-1	Uncultured bacterium	98%	LN649244
X16-1	<i>Brevundimonas</i> sp.	100%	LN681344
X16-2	<i>Burkholderia</i> sp.	100%	LN649249

36

37

38

39

40 **Table S3.** Shannon-Weiner index quantified based on the DGGE profile.

Samples	0d		7d		28d		56d		112d	
	CK	Crude	CK	Crude	CK	Crude	CK	Crude	CK	Crude
		oil		oil		oil		oil		oil
DG	3.23	3.4	3.13	2.86	3.33	3.38	3.45	3.51	3.44	3.16
SS	2.24	2.16	2.15	2.03	2.99	3.12	2.85	2.96	2.83	3.13
NE	3.85	3.36	3.39	2.52	3.22	2.74	3.46	2.53	3.43	2.66
XM	2.92	2.65	2.06	2.78	2.78	3.16	2.65	2.49	2.86	2.97

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

61

62 **Table S4.** Absolute numbers of copies of oil-degrading genes in soils from four regions of China.

Samples		DG	SS	NE	XM
Abundance of	0d	$(2.75 \pm 0.40) \times 10^4$	$(3.44 \pm 0.13) \times 10^5$	$(5.42 \pm 0.31) \times 10^5$	$(3.44 \pm 0.53) \times 10^4$
<i>AlkB</i> (copies/g,	7d	$(2.69 \pm 0.65) \times 10^5$	$(4.23 \pm 0.12) \times 10^5$	$(6.15 \pm 0.25) \times 10^5$	$(5.57 \pm 0.37) \times 10^4$
dm soil)	28d	$(1.51 \pm 0.46) \times 10^6$	$(3.40 \pm 0.57) \times 10^7$	$(3.23 \pm 0.21) \times 10^7$	$(2.90 \pm 0.35) \times 10^6$
	56d	$(2.73 \pm 0.26) \times 10^6$	$(5.79 \pm 0.43) \times 10^7$	$(6.33 \pm 0.42) \times 10^7$	$(1.70 \pm 0.53) \times 10^7$
	112d	$(2.30 \pm 0.16) \times 10^6$	$(6.29 \pm 0.32) \times 10^7$	$(7.39 \pm 0.34) \times 10^7$	$(2.48 \pm 0.32) \times 10^7$
Abundance of	0d	$(4.62 \pm 0.23) \times 10^3$	$(1.34 \pm 0.26) \times 10^3$	$(1.22 \pm 0.42) \times 10^3$	$(1.33 \pm 0.39) \times 10^3$
<i>Nah</i> (copies/g, dm	7d	$(2.83 \pm 0.53) \times 10^4$	$(3.72 \pm 0.43) \times 10^4$	$(2.26 \pm 0.51) \times 10^4$	$(7.85 \pm 0.57) \times 10^3$
soil)	28d	$(4.34 \pm 0.57) \times 10^4$	$(5.37 \pm 0.35) \times 10^5$	$(4.31 \pm 0.34) \times 10^5$	$(2.95 \pm 0.43) \times 10^5$
	56d	$(6.71 \pm 0.63) \times 10^4$	$(7.53 \pm 0.72) \times 10^5$	$(6.21 \pm 0.48) \times 10^5$	$(3.54 \pm 0.32) \times 10^5$
	112d	$(1.29 \pm 0.43) \times 10^5$	$(5.36 \pm 0.64) \times 10^6$	$(4.23 \pm 0.73) \times 10^6$	$(6.42 \pm 0.55) \times 10^5$
Abundance of <i>Phe</i>	0d	$(2.39 \pm 0.36) \times 10^3$	$(8.12 \pm 0.55) \times 10^3$	$(1.31 \pm 0.12) \times 10^3$	$(1.53 \pm 0.19) \times 10^3$
(copies/g, dm soil)	7d	$(1.68 \pm 0.25) \times 10^4$	$(1.39 \pm 0.55) \times 10^5$	$(9.40 \pm 0.24) \times 10^4$	$(2.36 \pm 0.17) \times 10^3$
	28d	$(5.54 \pm 0.54) \times 10^4$	$(9.67 \pm 0.25) \times 10^5$	$(1.30 \pm 0.97) \times 10^6$	$(9.40 \pm 0.13) \times 10^4$
	56d	$(8.91 \pm 0.32) \times 10^4$	$(3.12 \pm 0.75) \times 10^6$	$(4.47 \pm 0.42) \times 10^6$	$(4.72 \pm 0.42) \times 10^5$
	112d	$(1.14 \pm 0.47) \times 10^5$	$(2.99 \pm 0.23) \times 10^6$	$(3.49 \pm 0.42) \times 10^6$	$(5.32 \pm 0.26) \times 10^5$
Abundance of 16S	0d	$(6.52 \pm 0.34) \times 10^7$	$(2.85 \pm 0.54) \times 10^8$	$(2.57 \pm 0.48) \times 10^8$	$(1.52 \pm 0.29) \times 10^7$
rRNA (copies/g,	7d	$(4.34 \pm 0.12) \times 10^7$	$(2.65 \pm 0.21) \times 10^8$	$(9.40 \pm 0.64) \times 10^8$	$(1.10 \pm 0.22) \times 10^7$
dm soil)	28d	$(7.50 \pm 0.44) \times 10^7$	$(1.14 \pm 0.45) \times 10^8$	$(1.30 \pm 0.37) \times 10^9$	$(1.15 \pm 0.23) \times 10^8$
	56d	$(9.18 \pm 0.37) \times 10^7$	$(1.94 \pm 0.55) \times 10^9$	$(4.47 \pm 0.42) \times 10^9$	$(5.07 \pm 0.32) \times 10^8$
	112d	$(8.92 \pm 0.48) \times 10^7$	$(1.22 \pm 0.36) \times 10^9$	$(3.49 \pm 0.31) \times 10^9$	$(6.43 \pm 0.46) \times 10^8$

63

64

65

66

67

68

69

70

71

72 **Table S5.** Sequences, sizes of amplicons, and annealing temperatures of primer sets for qPCR.

Degradation genes	Proteins targeted		Sequence(5'-3')	Amplicon size (bp)	PCR annealing temperature(°C)	Reference
<i>AlkB</i>	Alkane monooxygenases	AlkB	AACTACMTCGA	100	50	1
		-F	RCAYTACGG			
		AlkB	TGAMGATGTGG			
		-R	TYRCTGTTCC			
<i>NahAc</i>	Naphthalene dioxygenase	NahA	ACTTGGTTCCGG	136	57	2
		c-F	AGTTGATG			
		NahA	CAGGTCAGCAT			
		c-R	GCTGTTGTT			
<i>Phe</i>	Phenol monooxygenase	Phe-F	GTGCTGAC(C/G)	206	49	3
			AA(C/T)CTG(C/T)			
			TGTTC			
		Phe-R	CGCCAGAACCA(C/T)TT(A/G)TC			
16S rDNA	Conservative region	16S-F	CGGTGAATACGT	140	55	4
			TCYCGG			
		16S-R	CGGTGAATACGT			
			TCYCGG			

73

74 **References**

- 75 1. Powell, S.M., Bowman, J.P., Ferguson, S.H. & Snape, I. The importance of soil characteristics
76 to the structure of alkane-degrading bacterial communities on sub-Antarctic Macquarie Island.
77 *Soil Biol Biochem* **42**, 2012-2021 (2010).
- 78 2. Park, J.-W. & Crowley, D.E. Dynamic changes in *nahAc* gene copy numbers during
79 degradation of naphthalene in PAH-contaminated soils. *Appl Microbiol Biot* **72**, 1322-1329

80 (2006).

81 3. Baldwin, B.R., Nakatsu, C.H. & Nies, L. Detection and enumeration of aromatic oxygenase
82 genes by multiplex and real-time PCR. *Appl Environ Microbiol* **69**, 3350-3358 (2003).

83 4. Suzuki, M.T., Taylor, L.T. & DeLong, E.F. Quantitative analysis of small-subunit rRNA genes
84 in mixed microbial populations via 5'-nuclease assays. *Appl Environ Microbiol* **66**, 4605-4614
85 (2000).

86

87

88

89

90

91

92

93

94

95

96

97

98

99

100

101

102

103

104

105

106

107

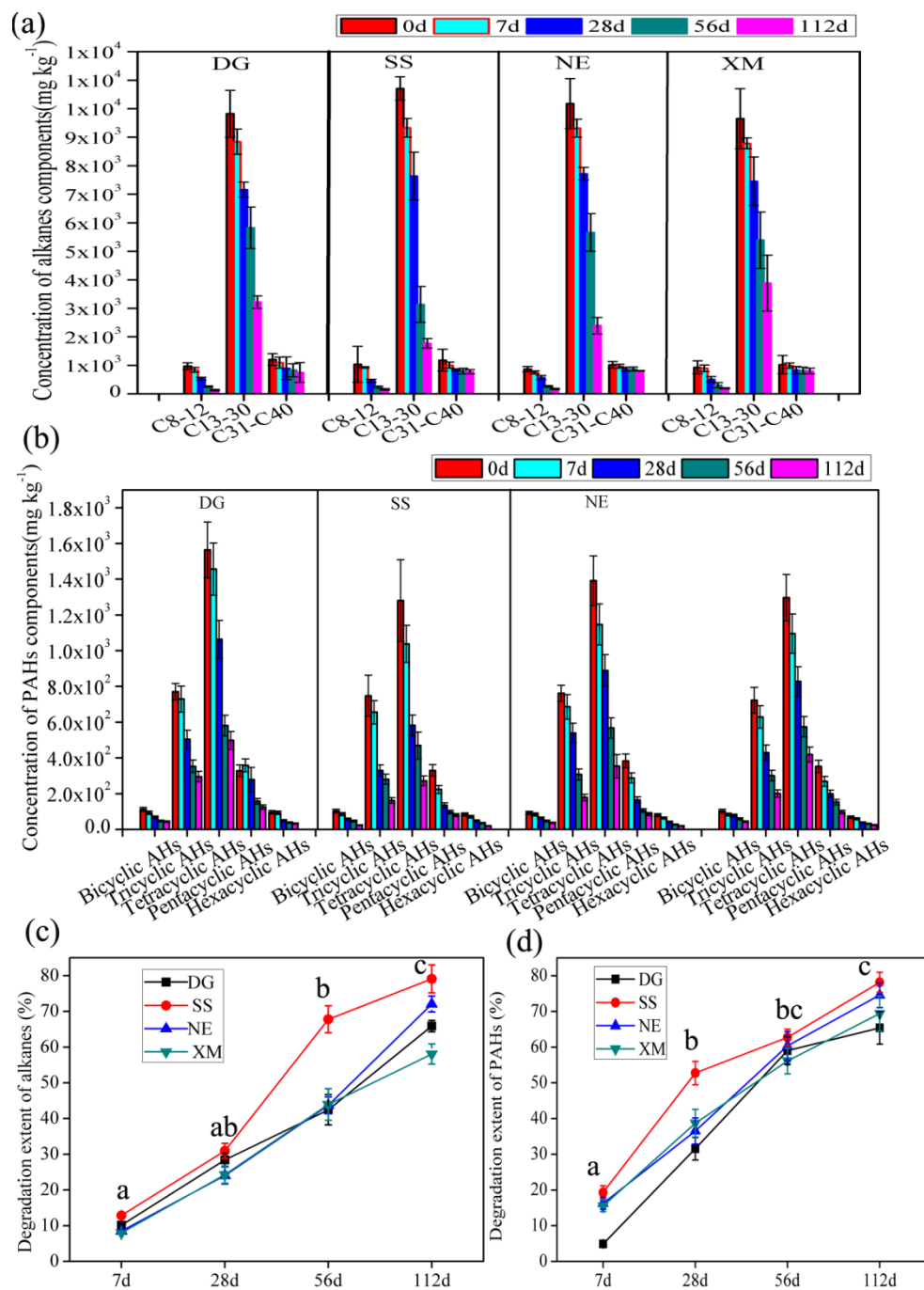
108

109

110

111

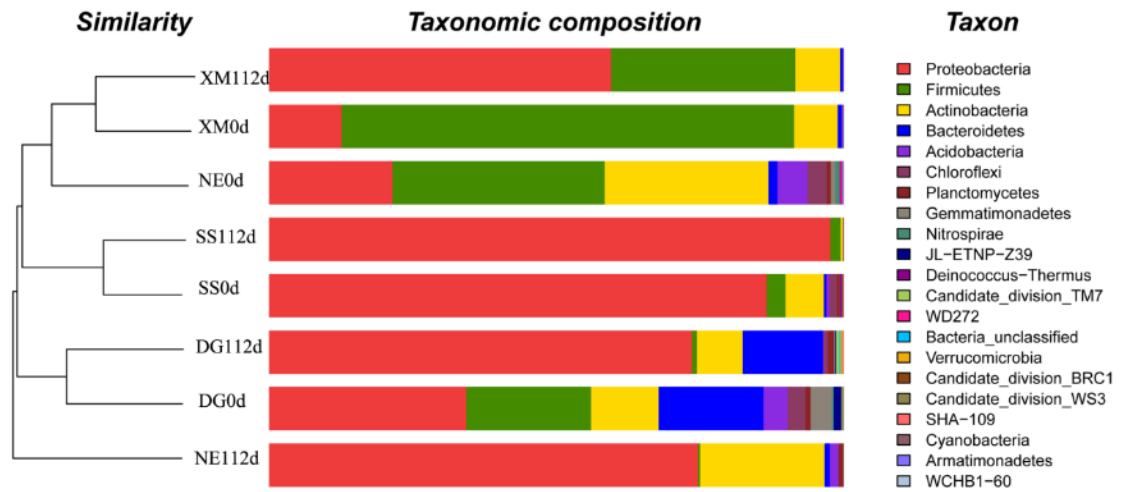
112



113

114 **Figure S1.** Residual concentrations and degradation of alkanes (C8-C40) and PAHs in soils quantified
 115 by use of GC-MS. (a) Concentrations of shorter-chain alkanes (C8-C12), medium-chain alkanes
 116 (C13-C30), and longer-chain alkanes (C31-C40). (b) Concentration of bicyclic, tricyclic, tetracyclic,
 117 pentacyclic, and hexacyclic PAHs. (c) Degradation extent of shorter-chain alkanes (C8-C12),
 118 medium-chain alkanes (C13-C30), and longer-chain alkanes (C31-C40). (d) Degradation extent of
 119 bicyclic, tricyclic, tetracyclic, pentacyclic, and hexacyclic PAHs.

120 Letters on the graph indicate a significant difference among different sampling times at $p < 0.05$
 121 according to Duncan's multiple range tests of One-Way ANOVA.
 122

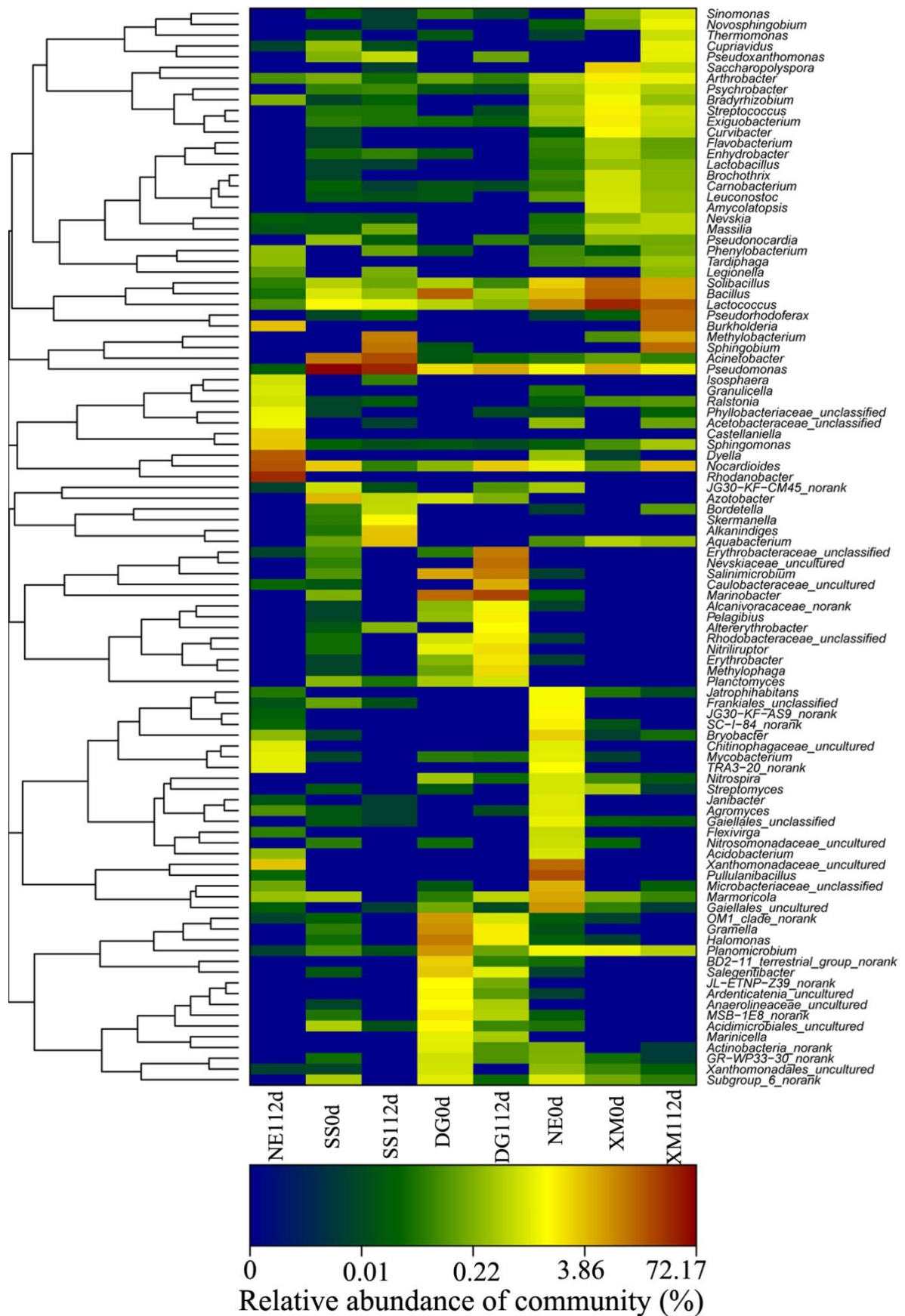


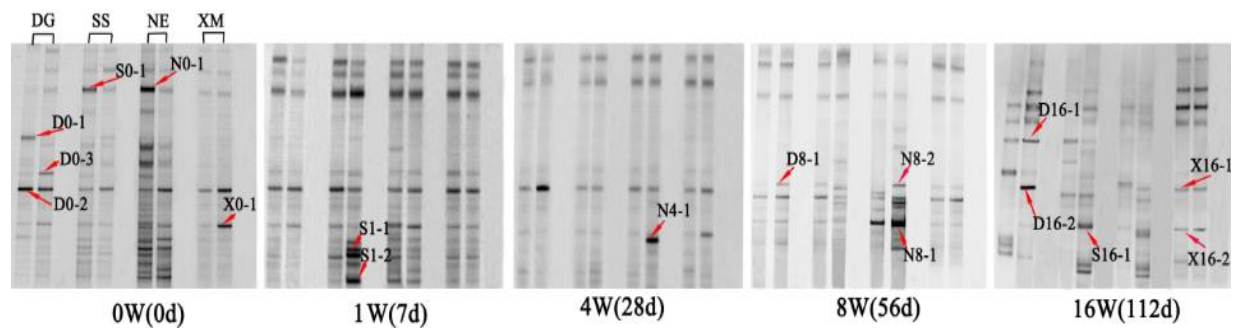
123 **Figure S2.** Results of cluster analysis and taxonomic composition of the microbial communities based
 124 on HTS. Mean proportions of bacterial phyla in studied soil groups. Phyla representing more than 2%
 125 of the bacterial community are summarized.
 126

127
 128
 129
 130
 131
 132
 133
 134
 135
 136
 137
 138
 139
 140
 141
 142

143

144





148

149 **Figure S4.** DGGE profile of soils from four typically geographical regions. DGGE bands collected

150 from four soils were of the same order of magnitude as the first picture. In each soil, two bands

151 represented blank control and with addition of 5% (by weight) crude oil, respectively. DG: Dagang

152 Oilfield; SS: Sea Sand; NE: Northeast China; XM: Xiamen.

153

154

155

156

157

158

159

160

161

162

163

164

165

166

167

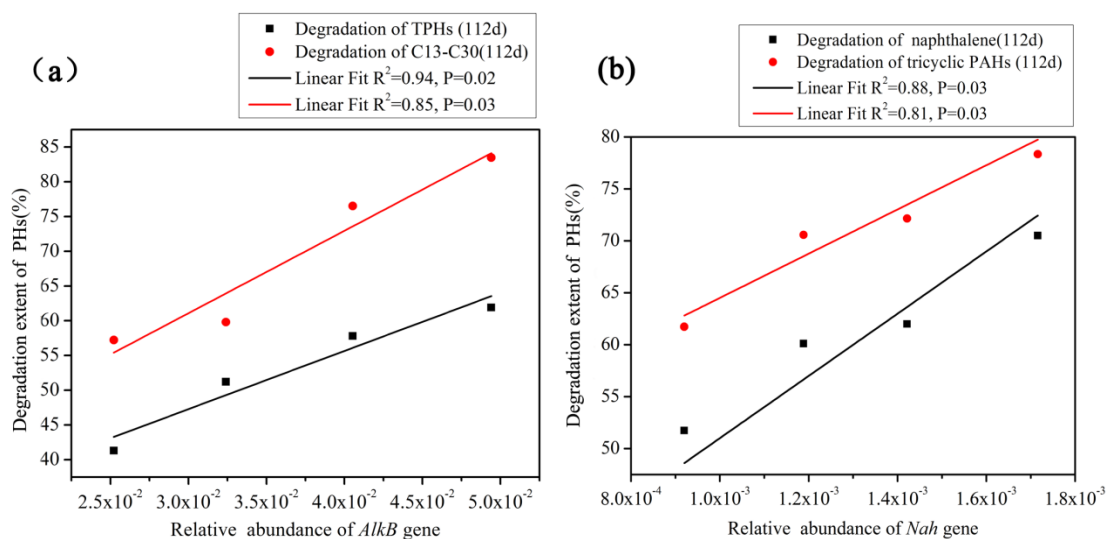
168

169

170

171

172



174

175 **Figure S5.** Pairwise correlations between metabolic gene abundances and PHs degradation. (a)176 Correlation between relative abundances of *AlkB* gene and oil degradation; (b) Correlation between177 *Nah* gene abundances and oil degradation.

178

179

180

181

182

183

184

185

186

187

188

189

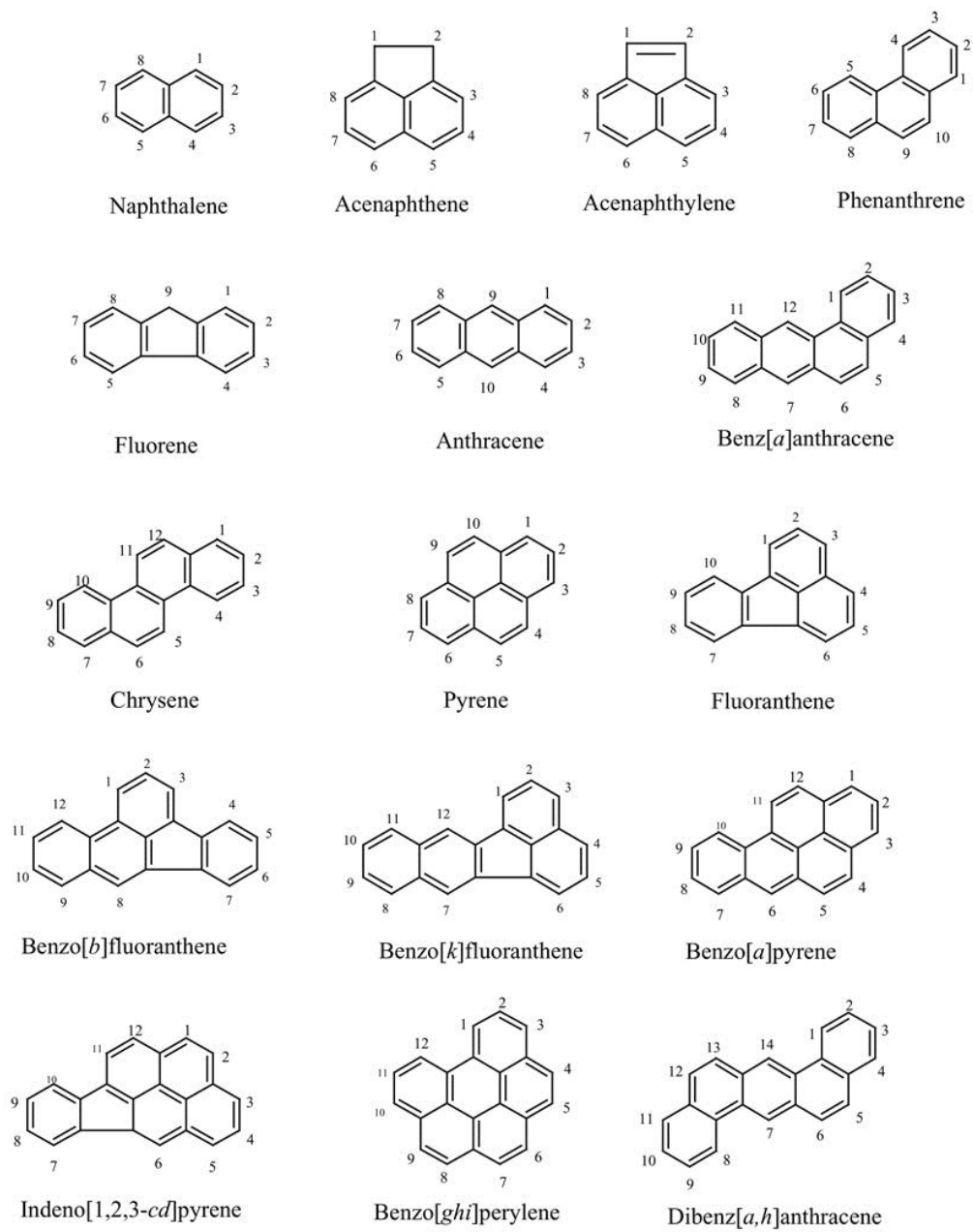
190

191

192

193

194



195

196 **Figure S6.** Structures and nomenclatures of the 16 PAHs on the EPA priority pollutant list.

197