1 **Title:**
2 Assessment of the petroleum hydrocarbon biodegradation potential of the sediment microbial community
3 from an urban fringing tidal marsh of Northern New England.
4
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14 **Running Title**: Diversity of fringing tidal marsh alkane degrading bacteria.
15

Abstract

We assessed the impact of dodecane, *n*-hexane and gasoline on the microbial diversity of chronically polluted fringing tidal marsh sediment from the Great Bay Estuary of New Hampshire. Dilution cultures containing saturated alkane concentrations were sampled at zero, one and 10 days, and *alkB* and *cyp153A1* alkane hydroxylase gene libraries and 16S rRNA sequences were analyzed. The initial sediment had the most diverse alkane hydroxylase sequences and phylogenetic composition whereas treated sediments became less functionally and phylogenetically diverse with alkane substrates apparently enriching a few dominant taxa. All 1- and 10-day samples were dominated by *Pseudomonas*-type alkane hydroxylase sequences except in dodecane treatments where primarily *Rhodococcus*-type alkane hydroxylases were detected. 16S rRNA profiling revealed that the Gammaproteobacteria, particularly *Pseudomonas,* dominated all one day samples, especially the *n*-hexane and gasoline treatments (63.2 and 47.2% respectively) and the 10-day *n*-hexane treatment (which contained 60.8% *Pseudomonas* and 18.6% *Marinobacter*). In contrast, the 10 days of dodecane treatment enriched for Actinobacteria (26.2% *Rhodococcus* and 32.4% *Mycobacterium*) and gasoline treatment enriched for Firmicutes (29.7%; mainly *Bacillus*, *Lysinibacillus* and *Rumelibacillus*). Our data indicate that fringing tidal marshes contain microbial communities with alkane-degrading abilities similar to larger meadow marshes, and support the hypothesis that alkane exposure reduces the functional and phylogenetic diversity of microbial communities in an alkane-specific manner. Further research to evaluate the ability of such fringing marsh communities to rebound to pre-pollutant diversity levels should be conducted to better assess the threat of petroleum to these habitats.

Keywords

Introduction

40
41   Salt marshes are frequently contaminated with petroleum through both large-scale releases, such
42 as the Deepwater Horizon well blowout in the Gulf of Mexico in 2010 (Atlas et al., 2015; Natter et al.,
43 2012; McGenity 2014), as well as chronic lower-level contamination from sources such as stormwater
44 and small marine transportation related spills (McGenity 2014; Vieites et al., 2004). Microbial
45 degradation of petroleum hydrocarbons released into marshes is the major means of removal, as
46 evidenced by research demonstrating both the loss of petroleum, and the expansion of hydrocarbon-
47 degrading taxa and associated degradative genes (Acosta-González et al,. 2015; Atlas et al., 2015;
48 Beazley et al., 2012; Kimes et al., 2014, Koo et al., 2015; Looper et al., 2013; Lu et al., 2012; Mahmoudi
49 et al., 2013; Vega et al., 2009; Zhu et al., 2004). Petroleum bioremediation potential is related to the
50 chemical composition of the polluting petroleum, the composition of the indigenous microbial
51 communities present in the receiving environment, and factors such as marsh vegetation (Acosta-
52 González et al., 2015; Atlas 1975; Atlas et al., 2015; Beazley et al., 2012). Petroleum contains hundreds
53 of different hydrocarbons, including high quantities of *n*-alkanes (Speight 1998). *N*-alkanes, are,
54 therefore, one of the major groups of contaminants that routinely intrude into salt marshes.
55   *N*-alkanes are some of the most biodegradable petroleum hydrocarbons, particularly under
56 aerobic conditions where many bacteria, particularly α, β and γ-Proteobacteria, Actinomycetales and
57 Firmicutes, can degrade a range of *n*-alkanes (Joye et al., 2016; Mason et al., 2012; Mukherjee et al.,
58 2017; Rojo 2009; Van Beilen & Funhoff 2007; Wang et al., 2010). Some alkane-degrading bacteria are
59 actually obligate or near-obligate alkanotrophs such *Alcanivorax borkumensis* (Sabirova et al., 2006).
60 Aerobic degradation of medium chain-length alkanes (C5-C16), is initiated by terminal carbon
61 hydroxylation (Van Beilen & Funhoff 2007), effected by two types of alkane hydroxylases: membrane-
62 bound non-haem diiron *alkB*-type hydroxylases (Kok et al., 1989; Van Beilen et al., 1994), and soluble
63 Class 1 cytochrome P450 Cyp153A1 (*CYP 153A1*) hydroxylases (Asperger et al., 1981; Kubota et al.,
64 2005; Maier et al., 2001). The *alkB* and *CYP 153A1* genes have been characterized in isolates and
65 environmental DNA and in the case of *alkB* are considered indicators of enhanced microbial hydrocarbon
66 degradation activity (Lu et al., 2012; Mukherjee et al., 2017; Van Beilen & Funhoff 2007, and others).
67 Some bacteria possess only *alkB*, others only *CYP 153A1*, and some have multiple alkane hydroxylase
68 systems that work in concert (Chen et al., 2017, Liu et al., 2011; Nie et al., 2014; Schneiker et al., 2006;
69 Van Beilen and Funhoff 2007; Wang et al., 2010a). In addition to *alkB* and *CYP 153A1* novel alkane
70 hydroxylases continue to be discovered (Guibert et al., 2016 and others).
71   The majority of studies on petroleum degradation by salt marsh microbial communities have
72 focussed on large scale release events due to shipping accidents or blowouts and large salt marsh meadow
73 systems such as those found in the Gulf of Mexico area impacted by the 2010 Deepwater Horizon
74 blowout (McGenity 2014). In New Hampshire, small estuarine marshes referred to as fringing marshes
75 comprise a significant amount of the total salt marsh habitat (Morgan et al., 2009, PREP 2013) which
76 have important ecosystem functions and values (Morgan et al., 2009). These fringing marshes are
77 different from large meadow marshes, possessing lower levels of organic carbon and plant density
78 (Morgan et al.,2009) and lower levels of denitrification enzyme activity (Wigand et al., 2004). Although
79 fringing marshes are the first location impacted by petroleum influx, which is considered one of the major
80 threats to such marshes in New Hampshire (NH DES, 2004), little is known about the petroleum
81 biodegradation potential of these marshes. In this study we aimed to assess the catabolic and phylogenetic
82 diversity of an alkane-degrading fringing marsh microbial community from a brackish chronically
83 petroleum-impacted fringing marsh site on the Cocheco River of the Great Bay Estuary of New
84 Hampshire (Watts et al.,2006). Furthermore we aimed to evaluate the response of the community to
85 different sources of n-alkanes (gasoline, *n*-hexane and dodecane). We hypothesized that given the chronic
86 nature of petroleum input in the area, the indigenous microbial community would contain diverse alkane
87 hydroxylase genes, and that exposure to different n-alkanes would select for different suites of alkane
88 hydroxylase genes, reducing gene-specific and overall phylogenetic diversity through selection of
89 degradative members of the community. To test this, we treated dilution cultures to each source of *n*-

3

90   alkanes for 10 days, analyzing *alkB* and *CYP 153A1* alkane gene fragments from clone libraries, and 16S
91   rRNA genes to assess changes in the functional and taxonomic structure of the community respectively.
92
93   **Materials and Methods**
94
95   **Sediment sampling and dilution cultures**
96
97   Sediment samples were collected from a tidal marsh vegetated with *Spartina alterniflora*, located along
98   the Cocheco River in Dover, NH (43°11'51.36"N 70°52'02.81"W) on 11 May 2011 (after spring plant
99   emergence) at low tide. The Cocheco River is listed as impaired (NHDES 2015) due to the chronic input
100   of petroleum hydrocarbon contamination from shipping and other sources (Magnusson et al.,2012) as
101   well as historical and current point and non-point sources including waste water treatment plants, landfill
102   leachate (such as the Superfund listed Tolend Road landfill in Dover NH: EPA) and urban stormwater.
103   Because the level of impervious surfaces is increasing (PREP 2013) stormwater-carried contaminants,
104   such as petroleum hydrocarbons (Brown et al.,2006; Makepeace et al.,1995), routinely enter the river and
105   its tidal marsh sediment. Several sediment samples were collected from the top 15 cm of a 3 m x 3 m area
106   containing areas vegetated and un-vegetated areas and composited into a sterile 1 gallon container. Large
107   plant material, rocks or other visible detritus was removed by hand and the sample was homogenized by
108   passing through a Food Mill (RSVP International Inc, Seattle, Washington) using two different filtering
109   disks with pore size 0.5 cm and 0.2 cm.   Dilution cultures were established by adding 1 g (wet weight)
110   sediment into 50 mL modified Minimal Salts Broth (Launen et al., 2008) in 250 mL Erlenmeyer flasks.
111   Dilution cultures received (separately) 1.5 mL gasoline (G) or *n*-hexane (H) in the vapor phase, dodecane
112   (D) neat to saturating concentrations or no alkanes (no-alkane control). All n-alkanes were maintained to
113   ensure saturating concentrations throughout the 10 days of exposure. Room temperature cultures were
114   shaken at 100 rpm to ensure aerobic conditions and were sampled at time 0 (initial, T0), and after 1 (T1)
115   and 10 (T10) days for genomic DNA extraction and subsequent processing.
116
117   **DNA extractions and clone library preparation and sequencing**
118
119   Genomic DNA was extracted from 4 mL of dilution cultures using the PowerSoil DNA Isolation
120   Kit (Mo Bio Laboratories, Carlsbad, CA). PCR was conducted using primers that targeted the *alkB* alkane
121   hydroxylase (Kloos et al.,2006; Van Beilen et al.,2006; Wang et al.,2010b). The forward primer of Kloos
122   et al.,was modified by addition of an R on the 3' end. PCR reactions were conducted using 1μM of
123   forward and reverse primers, 2X Green GoTaq Reaction Buffer (Promega, Madison, WI), 1 μL template
124   DNA (ca. 10 – 50 ng) and nuclease-free water (to 20 μL). The amplification cycle consisted of an initial
125   denaturing step of 95˚C for 5 minutes, followed by 35 cycles of 95˚C for 30 seconds, 55˚C for 30 seconds
126   and 72˚C for 30 seconds, and a final elongation of 72˚C for 5.5 minutes.
127   Hydroxylase gene fragments were cloned into pCR4-TOPO vectors (Life Technologies, Carlsbad,
128   CA). Plasmid DNA was isolated using the PureYield Plasmid Miniprep System (Promega). Sequencing
129   was conducted by the University of Washington High Throughput Genomics Center using either the M13
130   forward or reverse primers provided by the sequencing facility.
131
132   **Phylogenetic analysis of clone library sequences**
133

134   Primer sequences were removed from nucleotide sequences using Geneious v. 7.1.5 (created by
135 Biomatters). Sequences were aligned with a ClustalW alignment (in Geneious) and Phylip v. 3.695
136 (Felsenstein 2005). An alignment-associated DNA distance matrix was input into the program MOTHUR
137 v. 1.33.1 (Schloss & Handelsman 2009), that was used to cluster sequences into operational taxonomic
138 units (OTUs) based on the nearest neighbor algorithm and a 95% (nucleotide) sequence identity.
139 MOTHUR was also used to construct rarefaction curves for each library, and to calculate estimates of
140 nonparametric richness (Chao1 and Shannon-Weaver diversity indices) (Chao 1984; Chao and Lee 1992;
141 Pielou 1977). Phylogenetic trees were constructed from the ClustalW alignment of the derived amino acid
142 sequences by neighbor-joining analysis with 1000 bootstrap replicates using MEGA 5.0 (Tamura et
143 al.,2011).
144
145 **Nucleotide sequence accession numbers**
146
147   The nucleotide sequences reported in this study were deposited in the GenBank database with the
148 following accession numbers KT280353 to KT280397 (selected representatives of each *alkB* OTUs) and
149 KT305744 to KT305774 (selected representatives of each *CYP 153A1* OTUs).
150
151 **Quantification of relative *alkB*, *CYP 153A1* and *16S* rRNA gene abundance in dilution cultures**
152
153   The dominant *alkB* and *CYP 153A1* OTUs from the clone libraries were further analyzed using
154 real-time PCR. QPCR primers (Supplemental Table 1) were designed from the nucleotide sequences of
155 clones from within the selected OTUs using Primer Express (V3.0.1, Applied Biosystems). The
156 abundance of selected *alkB* and *CYP 153A1* OTU sequences was determined relative to the abundance of
157 bacterial 16S rRNA genes. Standard curves were generated using two representative plasmid clones from
158 each OTU. Plasmid DNA was linearized with the *NotI* restriction enzyme and quantified using a
159 Nanodrop 2000 (Thermo Orion). Standard curves for bacterial 16S rRNA genes were generated using
160 *Escherichia coli* JM109 genomic DNA. QPCR reactions were performed in 96-well Fast Optical
161 MicroAmp reaction plates (Life Technologies) where each reaction had a total volume of 12.5 µl: 1 µl of
162 environmental sample or plasmid DNA solution, 6.25 µl SYBR Green PCR Master Mix (Applied
163 Biosystems), 4.75 µl water and 0.25 µl of each primer. Thermal cycling and quantification were
164 performed using a StepOne Plus Real-Time PCR System (Applied Biosystems) instrument programmed
165 as recommended by the manufacturer (95°C for 10 min followed by 40 cycles of denaturation at 95°C for
166 30 s, primer annealing at the primer-specific annealing temperature (Table 3) for 1 min, and polymerase
167 extension at 72°C for 1 min. The final primer concentration was 0.2 mM. Amplification specificity was
168 achieved for each primer set, as determined by the presence of a single peak in each post-amplification
169 dissociation curve.
170
171 **Bacterial community sequencing**

172   PCR amplification of the 16S rRNA hypervariable region V6 was performed with a pool of
173 degenerate forward and reverse primers targeting bacteria as described by Huber at al., (2007). The 5′-
174 ends of the forward primers were fused with the Ion Torrent A-Adaptor plus a MID key sequence
175 (barcode), while the reverse primers were fused with the truncated P1-adapter sequence (trP1). The
176 primers were diluted in molecular grade laboratory water and pooled in equimolar concentrations for each
177 barcode set. Template from each treatment was amplified with a different barcode.

178   For amplicon library preparation purified genomic DNA from each treatment was amplified in 20
179 µL reactions as follows: 1 µL of DNA (10-50 µg/mL concentration), 10 µL 1X Phusion PCR master mix
180 (Thermo, Pittsburgh, PA), and 2 µL of 0.5 µM forward and reverse primers. The PCR conditions were:
181 94°C for 3 min, followed by 30 cycles of 94°C for 15 s, 58°C for 15 s, 68°C for 10 s, and a final
182 elongation step of 68°C for 30 s. Triplicate amplifications were performed for each treatment. PCR
183 products were run on 2% 1X TBE gels. Amplicons of the correct size were excised, pooled, and purified
184 with the Promega SV Gel and PCR Clean-Up kit (Promega, Madison, WI). Amplicon concentration was
185 estimated with the Qubit 2.0 instrument using the Qubit dsDNA HS assay (Life Technologies). The bar
186 coded amplicons were combined and diluted to 2.8×10^8 DNA/µL molecules per micro liter prior to
187 template preparation.
188   Sequencing template was prepared using the Ion OneTouch 200 Template Kit with the
189 corresponding protocol (Pub. Part no. 4474396 Rev. B, 20 Feb 2012). Sequencing of the amplicon
190 libraries were then carried out using the Ion Torrent Personal Genome Machine (PGM) system using the
191 Ion Sequencing 200 kit (from Life Technologies) and the corresponding protocol (Pub Part Number
192 4474596 Rev. C, 10 Oct 2012) on Ion Torrent 314 chips.

194 **Bacterial community sequence analysis**

196   Raw sequencing reads were processed using the Quantitative Insight Into Microbial Ecology
197 (QIIME) open source software package and the Virtual Box QIIME package (Caporaso et al., 2010a).
198 Raw sequences were filtered and screened for different quality criteria. Low quality reads were removed
199 as follows: i) all reads <70 b.p. were removed; ii) all reads with quality scores of lower than 20 were
200 removed; iii) no ambiguous reads were allowed; and iv) reads with homopolymers of 4 b.p. or less were
201 removed to reduce sequencing error. Chimeras rarely form in the V6 regions of the sequence due to its
202 small size (approximately 70- 200 b.p. sequence lengths) so the data were not screened for chimeras.
203   Subsequently, de novo OTUs were clustered at a 97% identity threshold and aligned to the
204 Greengenes database (DeSantis et al 2006) using PyNAST (Caporaso et al., 2010b). Alpha diversity was
205 calculated using the QIIME pipeline. The default metrics for the QIIME pipeline alpha diversity
206 measurement were edited to include the Shannon diversity index and phylogenetic distance, in addition to
207 Chao1 and observed species. The minimum number of sequences used for depth of coverage in alpha
208 rarefaction was set to 5000 for even sampling. Beta diversity metrics were also generated using QIIME.
209 5000 sequences were subsampled from each dilution sample for calculation of beta diversity using both
210 weighted and unweighted unifrac (Lozupone & Knight 2005) analyses. Principal Coordinate Analysis
211 (PCoA) (Vazquez-Baeza et al., 2013) and hierarchical clustering (UPGMA) (Caporaso et al., 2010a) were
212 utilized to visualize the data structure and differences between the samples. Jackknife replicates (100
213 replicates) were used to estimate the uncertainty in both the PCoA and hierarchical clustering plots.

215 **Results**

217 **Analysis of alkane hydroxylase clone libraries**

219 *alkB* **clone library**

221   *AlkB* alkane hydroxylase gene fragment clone libraries were prepared from genomic DNA
222 extracted from alkane-exposed and unamended cultures over 10 days. The distribution of OTUs, their

223   diversity, and matches to known sequences as determined by Blastx are shown in Table 1 and 2, and Fig.

224   1A and 2. Dilution culture alone reduced diversity of *alkB* gene fragments according to the Shannon-

225   Weaver diversity index and Chao1 estimates (Table 1) in the first day of dilution culture, however, both

226   measures increased by the T10 to levels greater than the initial T0 sediment indicating that diversity

227   rebounded in the dilution culture. In contrast, all alkane treatments reduced *alkB* gene fragment diversity

228   to levels less than those found in the no alkane control sample after 1 and 10 days of continuous alkane

229   exposure.

230   The taxonomic assignment of *alkB* sequences recovered from each sample library differed

231   according to treatment (Table 2, Fig. 1A). The initial sediment (the T0 NA sample), yielded 25 *alkB* clone

232   library sequences which contained 14 different OTUs, the majority of which were an 81% BlastX match

233   to *alkB* from *Pseudomonas aeruginosa* (OTU2) or an uncultured *Commomodaceae* sequence (OTU11)

234   (see Fig. 1A). After one day of dilution culture, the unamended control sample (T1 NA) yielded 14 clone

235   library sequences, containing 9 OTUs, most of which matched OTU2 (*P. aeruginosa*). By 10 days of

236   dilution culture alone (T10 NA) 26 *alkB* clone sequences were recovered, containing 15 OTUs, with the

237   majority matching OTU8 (uncultured/*Alcanivorax* sequences as best match) and OTU9 (*Kordiimonas*

238   *gwangyangensis*).

239   *AlkB* libraries prepared from *n*-hexane and gasoline amended samples yielded fewer OTUs

240   overall and possessed reduced diversity measures relative to the unamended control libraries (Table 1).

241   One day of exposure to both *n*-hexane (T1 H) and gasoline (T1 G) resulted in a selection for the *P.*

242   *veronii*-like OTU1 (Fig. 1A, 14/29 and 25/31 of the clone sequences from these libraries respectively).

243   This selection for *Pseudomonas*-type *alkB* sequences persisted into the 10 day samples where 18/24 (T10

244   H) and 16/21 (T10 G) were also sequences belonging to OTU1. Dodecane appeared to impact the *alkB*

245   diversity differently than exposure to *n*-hexane or gasoline. While the one day dodecane-exposed sample

246   was dominated by OTUs that match best to *Pseudomonas spp*. (Table 1 and Fig. 2A), by 10 days of

247   dodecane exposure *alkB* sequences were predominantly OTU4 (15/19 sequences recovered), which is a

248   99% BlastX match to an *alkB* from *Rhodococcus* sp. RP-11 (Table 2, Fig. 1A). OTU4 was the major

249   Gram positive type *alkB* identified in any *alkB* library prepared in this study. Overall, exposure to all of

250   the alkanes tested resulted in differing profiles of *alkB* clone sequences than observed in the no alkane

251   control samples, with *n*-hexane and gasoline treatment selecting for *alkB*s of *Pseudomonas*-type and

252   dodecane treatment selecting initially for *Pseudomonas*-type sequences with a switch to *Rhodococcus*-

253   type *alkB* sequences by day 10.

254   Rarefaction curves (Supplemental Fig. 1A) constructed for each *alkB* library indicated that all

255   libraries except the T0 and the 1- and 10-day unamended control libraries were adequately sampled (ie.

256   plateaus were observed

257

258   *CYP 153A1* **clone library analysis**

259

260   *CYP 153A1* clone libraries were constructed from amplicons from the initial sediment sample

261   (T0 NA), the no-alkane exposed control one and ten day samples (T1 NA and T10 NA), and the 1- and

262   10-day dodecane exposed samples (T1 D and T10 D), see Fig. 1B, 3 and Table 1. The other samples did

263   not yield sufficient *CYP 153A1* PCR product for library construction. Furthermore, the T1 NA and the T1

264   and T10 D libraries contained very few sequences (Table 1). The T0 initial sediment sample contained

265   sequences that assigned to 13 OTUs and had the highest Chao1 and Shannon Weaver scores (see Table

266   1). These sequences matched by BlastX to *CYP 153A1* from the alpha proteobacterial order *Rhizobiales,*

267   and genera *Sphingopyxis*, and *Parvibaculum,* as well as several uncultured bacteria that group with the
268   alphaproteobacteria (Fig. 3). The T1 NA library yielded only three sequences, each belonging to different
269   OTUs; one *Rhizobiales*-like sequence (OTU6), a *Rhodococcus* (OTU27) and *Parvibaculum*
270   *hydrocarboniclasticum* (OTU31). T10 NA contained predominantly *P. hydrocarboniclasticum* (OTU3)
271   and *Rhizobiales* (OTU2). The only sequence recovered from the one-day dodecane (T1 D) exposed
272   sample environment most closely matched a *CYP 153A1* from an uncultured *Rhizobiales* (OTU16) (Fig.
273   1B, Table 2). By 10 days of dodecane exposure most of the *CYP 153A1* sequences matched either *CYP*
274   *153A1* sequences from *Rhodococcus fasciens* (OTU1) or *Rhodococcus erythropolis* (OTU7).
275   Analysis of rarefaction curves (Supplemental Fig. S1B) constructed for each *CYP 153A1* library
276   indicated that only the 10-day exposure libraries (dodecane and no-alkane) reached a plateau and thus
277   were adequately sampled. Overall, the limited number of sequences recovered limits our ability to analyze
278   *CYP 153A1* alkane hydroxylases from the microbial community studied, although it is interesting that the
279   T10 D sample yielded a *Rhodococcus*-type *CYP 153A1* alkane hydroxylase sequence, similar
280   taxonomically to what was observed in the T10 D *alkB* clone library dataset.
281
282   **Quantification of relative *alkB* and *CYP153A1* gene abundance in dilution cultures**
283
284   QPCR was used to assess the relative abundance of the dominant alkane hydroxylase OTUs from
285   the sample libraries. Primers were designed to target al*kB* OTUs 1-5 and *CYP 153A1* OTUs 1,4 and 7
286   (Table 2) (primers are in Supplementary Table 1, results in Table 3). *AlkB* OTU5 was the only *alkB* OTU
287   detected in the T0 sample DNA and was also detected in all samples except for the 10 day unamended
288   control sample, suggesting that it may not be specifically induced by alkanes. The T1 H sample contained
289   very elevated OTU2 levels, a sequence which matches an alkane hydroxylase from *Pseudomonas*
290   *aeruginosa* (Table 2). OTUs 1 and 3 (also matches to *Pseudomonas*-derived alkane hydroxylases) were
291   also detected in this sample, although at lower levels. Ten days of hexane exposure (T10 H) resulted in
292   the decline of OTUs 2 and 3, and an approximately 7-fold increase in OTU1. In the gasoline-exposed
293   samples, OTUs 1, 3 and 5 were found at the same levels in the 1- day and 10- day samples. OTU2 was
294   not detected in the 1- day sample but was present at relatively high abundance in the 10- day gasoline-
295   exposed sample (T10 G). The 1-day dodecane exposed sample contained detectable *alkB* OTUs 1, 2, 3
296   and 5 and no detectable OTU4. By day 10, however, OTU4 dominated. Overall, the *alkB* qPCR analysis
297   supported the clone library analysis, demonstrating a selection for *Pseudomonas*-type *alkBs* in response to
298   *n*-hexane and gasoline treatment, and for *Rhodococcus*-type *alkBs* in response to dodecane treatment. The
299   *CYP 153A1* qPCR analysis was largely unsuccessful, with no detection of any of the selected OTUs other
300   than the OTUs 1, 4 and 7 in the 10- day dodecane-exposed sample which may suggest *CYP 153A1* type
301   alkane hydroxylases were not important in alkane degradation in this system or because of the limited
302   data, suggested that more extensive sampling and analysis may be required.
303
304   **16S rRNA sequence analysis**
305   Changes in the bacterial community in response to different alkanes were examined by tag
306   sequencing of the V6 region of the 16S rRNA using an Ion Torrent PGM. A total of 321,310 reads were
307   obtained, of which 281,782 passed quality filtering and were mapped to individual barcodes using the
308   QIIME pipeline. The number of sequences mapped to each treatment ranged from a high of 68,610 in the
309   initial sediment (T0) sample to a low of 92 sequences in the day 10 unamended control treatment (T10
310   NA) (Table 4). The processed data were then used to calculate alpha (Shannon, Chao1, phylogenetic

311   distances and PCoA analyses; Table 4 and Fig. 6) and beta (Unifrac analysis; Fig. 5) diversity metrics at a
312   sampling depth of 5000 with T10 NA excluded due to the low read number (Table 4).

313   Between 93.6 % and 99.5 % of the processed sequences could be classified below the bacterial
314   root using the QIIME pipeline with the majority of sequences classified to the family or genus level.
315   OTUs were divided into 13 groups for ease of analysis (Fig. 4), with each Group assigned to a genus,
316   family or Class, as could be achieved by our analysis. Group I comprised the sequences that could not be
317   classified and accounted for between 0.5 and 6.4 % of all sequences obtained. Group XIII represents all
318   rare OTUs (ie. each sequence type is ≤ 1 % of the sequences from a given sample) pooled into Group
319   XIII. The taxonomic annotation for the remaining groups is included in the Fig. 4 legend.

320   The initial sediment bacterial community (T0) was the most diverse sample by all measures other
321   than the Chao1 score (Table 4) with the most abundant OTU identified as Group XII (*Pseudomonas*:
322   3.2%). The T0 sample contained some sequences not found in any other sample, such as *Geobacter* (2.8
323   %), *Clostridium* (2.8 %), *Flavobacterium* (2.4 %) and *Burkholderiales* (10%). The Gram positive
324   sequences were diverse and represented 6.5% of the total. Dilution alone (culture in the absence of alkane
325   addition), reduced diversity and altered the community composition as seen in other sections (see
326   Discussion). The T1 NA sample was less diverse than the T0 sample by all measures calculated (Table 4)
327   and contained a community dominated by Gammaproteobacteria (84.7%) mainly *Acinetobacter* (Group
328   XI: 43.5 %) and *Pseudomonas* (Group XII: 26.9%). The T10 NA sample was dominated by
329   Gammaproteobacteria (22.8%), of which 8.7% were Group IX *Marinobacter*. This sample could not be
330   assessed for diversity metrics as it contained only 92 reads.

331   While our assessment of community diversity is limited by the lack of ability to calculate
332   diversity measures on the 10-day no alkane control (T10 NA), the one day analysis indicates that *n*-
333   hexane and gasoline reduced the diversity of the communities sampled beyond the loss of diversity seen
334   in one day of dilution alone (the no alkane control, T1 NA). Interestingly, this was not the case with
335   dodecane exposure, which instead resulted in an increase in diversity by all measures after one day of
336   exposure (T1 D). Comparing the diversity measures in all three types of alkane exposure after 10 days of
337   exposure (T10 D, T10 H and T10 G) indicates that diversity continued to decline in the *n*-hexane and
338   gasoline exposed treatments while it declined to a lesser degree in dodecane exposure. Hierarchical
339   clustering (Fig. 5) from unweighted and weighted unifrac analyses revealed the dodecane-exposed
340   samples clustered with the initial T0 sample, separate from all other samples. PCoA analysis (Fig. 6)
341   revealed that the similarities between the different hydrocarbon amendments and the no-hydrocarbon
342   control dilution culture were driven mainly by the *Gammaproteobacteria*.

343   One day of exposure to *n*-hexane and gasoline shifted the community to predominantly
344   Gammaproteobacteria, especially *Pseudomonas* (Group XII) and Gammaproteobacteria (Group VIII).
345   This Gammaproteobacterial dominance persisted to 10 days in the *n*-hexane exposed culture, but in the
346   gasoline-exposed culture the proportion of *Gammaproteobacteria* declined by 10 days and an increase in
347   *Alphaproteobacteria*, specifically the family *Acetobacteraceae* (Group VII: 22.5%) occurred. The
348   proportion of Gram positive bacteria increased in the T10 G sample to 29.7%, predominantly Firmicutes
349   from the genera *Bacillus*, *Lysinibacillus* and *Rumelibacillus*. As observed in the analysis of *alkB* clone
350   sequences, dodecane exposure caused a transient shift towards Gammaproteobacteria (56.2%) after one
351   day of exposure but by 10 days of continued dodecane exposure the *Gammaproteobacteria* declined (to
352   3.1% of sequences), and an upsurge in Gram positive bacteria (to 59.0%) occurred. The Gram positive
353   bacteria were mainly from the class *Actinobacteria*, chiefly *Mycobacterium* (Group II: 32.4 %) and
354   *Rhodococcus* (Group III: 26.2 %). Overall, the T10 D sample contained the greatest proportion of Gram
355   positive sequences observed in any sample (The T0 NA sample contained 6.5%, all T1 samples contained
356   less than one percent, T10 H contained 3.9%, T10 G 29.7% and T10 D 59.0%).

357   In total, the analysis of the microbial community agrees with the *alkB* clone library analysis,
358   indicating that Gammaproteobacteria are important members of the microbial community that respond
359   quickly to both dilution and alkane exposure, although the specific chemical nature of the alkane present

360 influences the community response. The ability of gasoline, and dodecane exposure, to recruit Gram
361 positive response is also noted.
362
363 **Discussion**
364
365   Small brackish estuarine tidal marshes, referred to as fringing marshes, are an important coastal
366 habitat in New Hampshire (Morgan et al., 2009 and references therein, PREP 2013), providing essential
367 ecosystem services similar to larger meadow marshes. These fringing marshes are under pressure from
368 sea level rise and exposure to pollutants such as petroleum (Morgan et al., 2009; NHDES 2004; PREP
369 2013; Stralberg et al., 2011). Unlike larger meadow marshes, relatively little is known about the ability of
370 fringing marsh microbial communities to degrade petroleum hydrocarbons, knowledge that would help us
371 better predict marsh resilience. In this study. we assessed the n-alkane degrading potential of a fringing
372 marsh microbial community from the Cocheco River of the Great Bay of New Hampshire, a site where
373 the role of *Spartina alterniflora* in phytoremediation of PAHs has been documented (Watts et al., 2006).
374   By focusing on alkane hydroxylase genes (*alkB* and *CYP 153A1*) the functional diversity was
375 assessed in the initial sediment, and dilution cultures treated with *n*-hexane, gasoline, dodecane or dilution
376 alone (no alkanes) for 10 days. The initial community was already enriched in *Pseudomonas*-type *alkB*
377 sequences which increased in relative abundance by one day's exposure to dilution alone, and all three
378 sources of alkanes tested. The initial dominance and rapid expansion of Gammaproteobacteria, especially
379 *Pseudomonas spp.* has been shown in a variety of environments including meadow salt marshes (Acosta-
380 Gonzales et al., 2015; Atlas et al., 2015; Beazley et al., 2012; Kasai et al., 2001; Koo et al., 2015; Kostka
381 et al., 2011; Lamendella et al., 2014; Liu & Liu 2013, Looper et al., 2013). By 10 days of further culture
382 we observed a differential response according to treatment condition, with gasoline and *n*-hexane further
383 enriching the fraction of *Pseudomonas*-type *alkBs* and reducing the overall diversity of *alkBs* recovered to
384 a greater extent than that seen in the no alkane control or the dodecane exposed cultures. In contrast,
385 dodecane exposure enriched for *Rhodococcus*-like *alkB* sequences and resulted in a 10- day community
386 with slightly more diverse *alkBs* than seen in the no alkane control. Interestingly we were only able to
387 amplify *CYP 153A1* -type genes from the no-alkane control and dodecane dilution cultures and failed to
388 detect *CYP 153A1* genes in most samples using QPRC (Table 1, Supplemental Table 1). This may
389 suggest that *CYP 153A1* alkane hydroxylases are not as important as *alkB* type hydroxylases in the
390 fringing marsh we studied, which contrasts with other reports focusing on petroleum-degrading bacteria
391 in marine and soil environments (van Beilen et al., 2006, Wang et al., 2010a). The role (or lack of role) of
392 CYP 153A1 type alkane hydroxylases in fringing salt marsh microbial communities requires further
393 study.
394   Analysis of the 16S rRNA V6 region to assess the phylogenetic diversity and taxonomic
395 composition of the whole community generally supported the findings of the clone library functional
396 analysis. The initial sediment community was highly diverse, with the dominant taxa present (3.2%)
397 matching to *Pseudomonas* spp. All dilution cultures, including the no-alkane control, exhibited a decrease
398 in diversity, richness and phylogenetic distance (PD) after one day of dilution culture (Fig. 4, Table 4)
399 which is expected given that dilution alone reduces diversity (Franklin et al., 2001, Hewson et al., 2003,
400 Schaffer et al., 2000, Yan et al., 2015). Comparing the diversity values obtained after one day of
401 treatment (we were not able to compare diversity values at 10 days of treatment due to the low read
402 number in the 10-day no alkane control), and the 10 day cultures, indicated that exposure to gasoline and
403 *n*-hexane reduced diversity to a greater degree than seen in the dodecane treatment or the no alkane

404 control treatment. This agrees with our functional gene analysis and the findings of other studies
405 documenting the negative impact of highly concentrated petroleum hydrocarbons on microbial diversity
406 (Acosta-González et al., 2015; Atlas et al., 2015; Beazley et al., 2012; Guibert et al., 2012; Mason et al.,
407 2012; Wang et al., 2011; Pérez-de-Mora et al., 2011; Saul et al., 2005). Others have reported a rebound in
408 diversity once petroleum hydrocarbon concentrations decline (through degradation: Atlas et al., 2015;
409 Koo et al., 2015; Paisse et al., 2010). Given that we maintained saturating concentrations of n-alkanes,
410 further study would be required to determine if the sediment microbial community of the fringing marsh
411 we studied is as resilient as larger marshes appear to be in the face of petroleum influx (Acosta-González
412 et al., 2015; Atlas et al., 2015 and others).
413   The taxonomic composition of the entire microbial community varied by treatment, generally
414 supporting the taxonomic assignment of *alkB* genes recovered. Gammaproteobacteria, particularly the
415 rapidly responding *Pseudomonads*, dominated in the initial sediment and were enriched in all one day
416 dilution cultures (alkane-amended or unamended) (Fig. 4), as well as in the 10 day gasoline and *n*-hexane
417 exposed cultures. Gammaproteobacteria are known to respond rapidly to dilution alone (Yan et al., 2015),
418 as well as being important early responders in petroleum-contaminated sites including larger salt marshes
419 (references above).
420   Interestingly, by 10 days of exposure to dodecane and gasoline, there was an increased proportion
421 of Gram positive bacteria, with the T10 D sample containing 59.0% Gram positive sequences mainly
422 from the genera *Rhodococcus* (26.2%) and *Mycobacterium* (32.4%), while the T10 G sample was
423 dominated by Firmicutes (29.7%), mainly from the genera *Bacillus*, *Lysinibacillus* and *Rumelibacillus*.
424 *Rhodococcus* species are known alkane degraders (Hassanshahian et al., 2010, Larkin et al., 2005, Liu &
425 Liu, 2013, Sekine et al., 2006, Van Hamme & Ward 2001, Whyte et al., 1998 & 2002, Xu et al., 2007)
426 documented in petroleum-impacted salt marshes (Acosta-González et al., 2015; Mahmoudi et al., 2013,
427 Liu & Liu 2013), as are Mycobacterium (Atlas et al., 2015 and others). The role of Gram positive bacteria
428 in petroleum degradation has been established in larger salt marshes and other systems (Acosta-González
429 et al., 2015; Atlas et al., 2015; Koo et al., 2015; Launen et al., 2008; Looper et al., 2012; Liu & Liu 2013;
430 Mahmoudi et al., 2013) and there is evidence that Gram positive bacteria may be important in the
431 secondary response to petroleum degradation, increasing in abundance more slowly than the rapidly
432 responding Gammaproteobacteria (Koo et al., 2015). Our results suggest that the role of Gram positive
433 bacteria in responding to alkanes in fringing marshes may be similar.
434   Selection of certain microbial community members over others when challenged with different
435 types of n-alkanes may reflect differences in alkane-metabolizing enzymes, or physiological differences
436 that affect the update or other aspects of alkane metabolism between microbes (Grund et al., 1975; Larkin
437 et al., 2005; Smits et al., 2002; Van Beilen et al., 1994; Van Beilen et al., 2005; Wang & Shao 2013;
438 Wentzel et al., 2007), alkane toxicity (Ramos et al., 2002; Vermuë et al., 1993; Walker & Colwell 1975),
439 alkane uptake from the environment (Bouchez-Naïtali et al., 1999; Rosenberg & Rosenberg 1981; Van
440 Hamme & Ward 2001), biosurfactants (Desai & Banat 1997; Ivshina et al., 1998; Pornsunthorntawee et
441 al.,2008, Pi et al.,2017) or relative growth rates (Vieira-Silva & Rocha 2010). Resolving the mechanisms
442 underlying the selection for specific community members by specific alkanes, as we observed here,
443 requires further study, likely involving a culture-based approach.
444   Overall, our results indicate that the fringing marsh studied contains a diverse microbial
445 community enriched with *Pseudomonas*-type alkane degrading bacteria able to expand rapidly when
446 exposed to environmental change (dilution and alkane exposure), as has been observed in larger meadow-
447 type salt marshes elsewhere (Acosta-González et al., 2015; Beazley et al., 2012; Hazen et al., 2010;

11

448   Mason et al., 2012). The importance of Gram positive bacteria, especially *Rhodococcus,* in a slower

449   response to dodecane exposure was also observed, and agrees with findings in meadow marshes and other

450   environments (Acosta-González et al., 2015; Atlas et al., 2015; Mahmoudi et al 2013, Liu & Liu 2013).

451   Overall, our data suggest that the microbial community studied is capable of responding to perturbation

452   by alkanes, and that it does so in an alkane-specific manner, supporting the potential of such fringing

453   marshes to buffer against petroleum influx through degradation. Given the importance of fringing

454   marshes in the New England region, and the threat of petroleum influx into these marshes, it seems likely

455   that more study is merited. Two areas of particular relevance would be an evaluation of the ability of

456   these communities to degrade whole-oil mixtures, such as home heating oil (which is regularly

457   transported in the Great Bay Estuary), as well as an assessment of the ability of these communities to

458   recover diversity once petroleum has been degraded.

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469   **References**

470

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721   **Table 1.** Diversity analysis of functional gene clone libraries. OTUs were clustered at the 95% identity
722   level using the furthest neighbor in Mothur version 1.33.3

Library	N	N	H'	Chao1
alkB				
TO NA	25	14	2.44	23
T1 NA	14	9	2.04	14
T1 H	29	8	1.75	8
T1 D	33	9	1.81	19
T1 G	31	4	0.65	5
T10 NA	26	15	2.46	42.5
T10 H	24	2	0.56	2
T10 D	19	3	0.66	3
T10 G	21	5	0.87	6.5
CYP 153A1				
TO NA	13	13	2.56	91
T1 NA	3	3	1.1	1
T1 D	21	1	0	6
T10 NA	31	10	1.85	13.3
T10 D	1	5	1.13	5.5

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724   [a]*N*, number of clones sequenced from each library; *n*, number of OTUs observed; H', Shannon-Weaver
725   diversity index, Chao1 nonparametric richness estimate. Numbers in parentheses represent 95%
726   confidence intervals; ND, not determined.

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Table 2. Best BlastX matches (by identity) in the GenBank database for the dominant alkane hydroxylase clone OTUs. Where best match has no taxonomic information the first match with taxonomic information is also included.

OTU # | # of sequences in OTU[1]	Gene ID	Name	Identity (%)	e-value
alkB				
OTU1|84	WP_017846506.1	*Pseudomonas veronii*	100	3E-114
OTU2|16	WP_026651424.1	*Pseudomonas aeruginosa*	81	3E-75
OTU3|15	WP_026651424.1	*Pseudomonas aeruginosa*	84	3E-87
OTU4|15	ABB90689.1	uncultured bacterium	99	8E-116
	AID55567.1	*Rhodococcus* sp. RP-11	99	3E-115
OTU5|12	CDI44588.1	*Pseudomonas lini*	96	6E-113
OTU6|7	WP_038843879.1	*Pseudomonas fluorescens*	99	2E-113
OTU7|6	ACT91140.1	uncultured bacterium	100	1E-119
	ADG26619.1	uncultured *Rhizobiales bacterium*	100	2E-119
OTU8|5	AGQ20900.1	uncultured bacterium	80	9E-91
	ACJ22711.1	*Alcanivorax* sp. S15-9	67	3E -80
OTU9|5	ACJ22725.1	*Kordiimonas gwangyangensis*	81	5E-85
OTU11|4	AGQ20978.1	uncultured bacterium	83	3E-95
	WP_027103373.1	*Comamonadaceae bacterium URHA0028*	84	7E-94
OTU13|3	AGQ20902.1	uncultured bacterium	82	3E -90
	WP_027103373.1	*Comamonadaceae* bacterium URHA0028	81	7E -89
OTU16|2	ABB96070.1	uncultured bacterium	86	1E -96
	WP_027103373.1	*Comamonadaceae* bacterium URHA0028	85	1E-93
OTU18|2	WP_030535917.1	*Rhodococcus erythropolis*	100	4E-112
OTU19|2	BAG06232.1	*Rhodococcus erythropolis*	99	7E-117
CYP 153A1				
OTU1|13	CCO96780.1	uncultured bacterium	100	4E-64
	WP_032375608.1	*Rhodococcus fascians*	100	3E-63
OTU2|11	ADF27209.1	uncultured *Rhizobiales* bacterium	93	2E-60
OTU3|8	AKG58848.1	uncultured bacterium	88	2E-56
	ADO15009.1	*Parvibaculum hydrocarboniclasticum*	80	7E-51
OTU4|4	ADF27251.1	uncultured Rhizobiales bacterium	93	2E-61
OTU5|3	CCO96917.1	uncultured bacterium	98	4E-63
	ACP39704.1	*Parvibaculum* sp. S18-4	97	7E-62
OTU6|2	ADF27232.1	uncultured *Rhizobiales* bacterium	94	3E-48
OTU7|2	CAH56117.1	*Rhodococcus erythropolis*	99	2E-64
OTU16|1	CCO96881.1	uncultured bacterium	86	5E-55
	ADF27267.1	uncultured *Rhizobiales* bacterium	99	2E-53
OTU27|1	ABI14020.1	*Rhodoccus* sp. MOP100	87	1E-54
OTU31|1	AKG58846.1	uncultured bacterium	78	1E-49
	ADO15009.1	*Parvibaculum hydrocarboniclasticum*	77	5E-49

731 [1] The number of matches to this OTU relative to all of the clone sequences from all libraries is shown. For

732 example, there were 84 clone library sequences that comprised OTU1 when all libraries were considered.

733 **Table 3.** QPCR analysis of selected alkane hydroxylase OTU DNA. Standard deviations are included in

734 parentheses.

OTU	Abundance of OTU as:			Copy # of OTU DNA/ng gDNA Copy # 16S rDNA/ng gDNA					
	Sample[a]								
AlkB									
	T0	T1 NA	T10 NA	T1 D	T10 D	T1 H	T10 H	T1 G	T10 G
1	ND	ND	ND	0.05 (±0.08)	0.02 (±0.02)	0.40 (±0.33)	3.00 (±2.01)	0.33 (±0.27)	0.48 (±0.55)
2	ND	1.30 (±0.83)	ND	0.21 (±0.29)	ND	1009 (±873.83)	0.02 (±0.02)	ND	71 (±113.85)
3	ND	0.74 (±0.42)	ND	2.36 (±1.18)	0.64 (±0.14)	2.88 (±1.49)	0.59 (±0.18)	0.04 (±0.01)	0.08 (±0.00)
4	ND	ND	ND	ND	0.57 (±1.79)	ND	ND	5.95 (±14.61)	ND
5	0.05 (±0.01)	0.03 (±0.02)	ND	0.02 (±0.02)	0.03 (±0.01)	0.06 (±0.04)	0.01 (±0.01)	0.03 (±0.01)	0.02 (±0.03)
CYP 153A1									
1	ND	ND	ND	ND	0.47 (±0.68)	ND	ND	ND	ND
4	ND	ND	ND	ND	0.10 (±0.23)	ND	ND	ND	ND
7	ND	ND	ND	ND	0.31 (±0.81)	ND	ND	ND	ND
16	ND	ND	ND	ND	ND	ND	ND	ND	ND

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736 [a] Sample names are given at times of sampling followed by type of alkane where ND = not detected. T =

737 time of exposure (days), NA = no alkanes (control), D = dodecane, H = *n*-hexane, G = gasoline.

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743 **Table 4**. Summary of IonTorrent PGM 16S rDNA V6 tag sequencing efforts and alpha diversity metrics.
744 Standard deviations are included in parentheses.

Library	Barcode	Processed sequences	OTUs observed	Chao1	Shannon Index	Phylogenetic Distance
T0	ATCAG	68,610	2132	4942 (216)	10.0 (0.01)	75
T1 NA	GTGAG	23,508	528	1551 (201)	5.0 (0.1)	34
T1 H	GATCT	45,242	426	1274 (145)	4.4 (0.0)	28
T1 D	CGTCT	13,730	1736	5274 (344)	8.4 (0.0)	61
T1 G	CGACG	62,669	346	921 (70)	3.9 (0.0)	23
T10 NA	AGCAT	92	nd	nd	nd	nd
T10 H	TGTCA	9,794	407	407 (38)	2.5 (0.0)	16
T10 D	GCGAT	32,439	782	2075 (246)	5.5 (0.0)	42
T10 G	TCTGT	25,689	872	872 (106)	4.5 (0.0)	22

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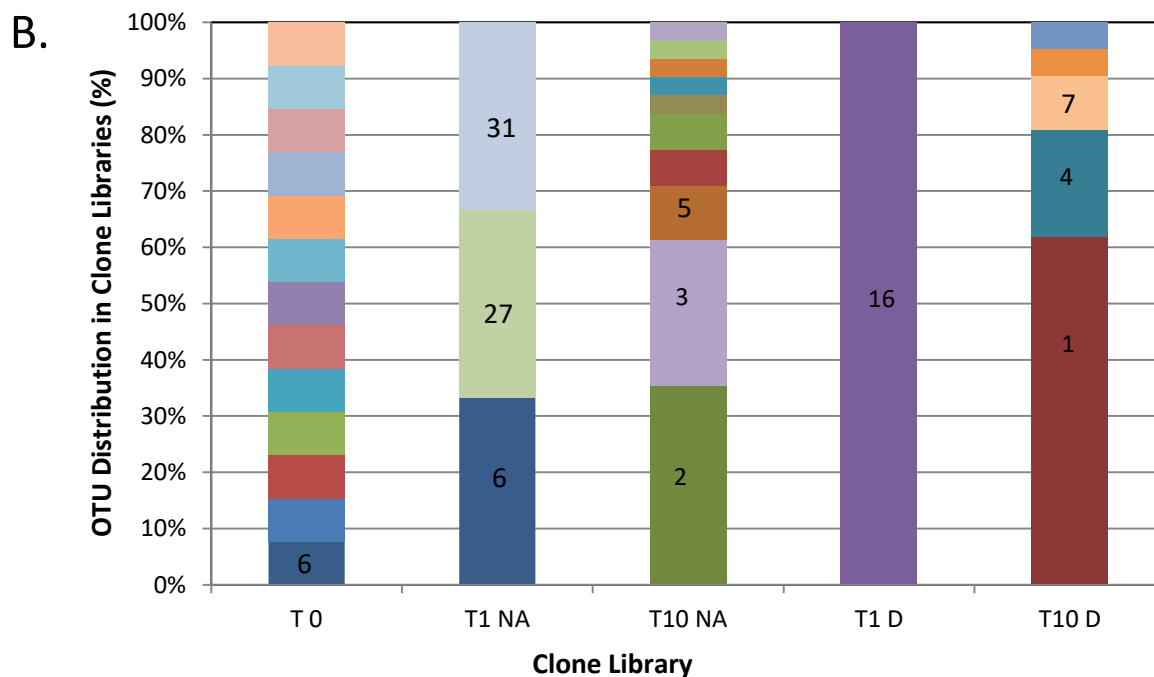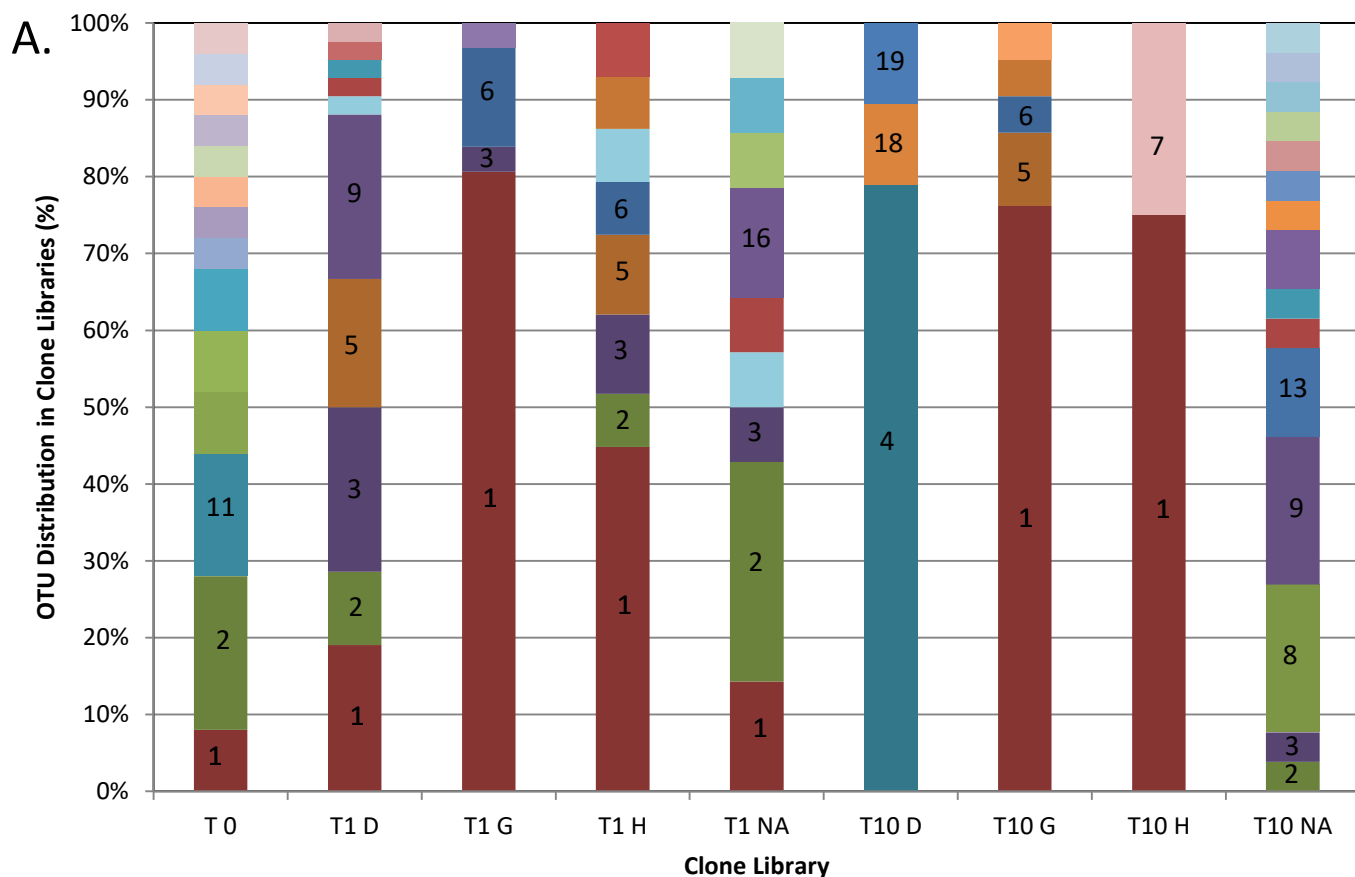
Figure 1. The distribution of *alkB* (A) and *P450* (B) OTUs in clone libraries derived from initial (T0), 1 day (T1) and 10 day (T10) samples exposed to dodecane (D), gasoline (G), *n*-hexane (H) or no alkanes (NA – control). Alignments, generation of distance matrices and clustering into OTUs at a similarity level of 95% was performed as described in the Methods. OTU numbering is included for OTUs that occurred in >10% of the total recovered for that library. See Table 2 for further information on OTU identity and Table 1 for the number of clone sequences in total for each library shown.

Fig. 2
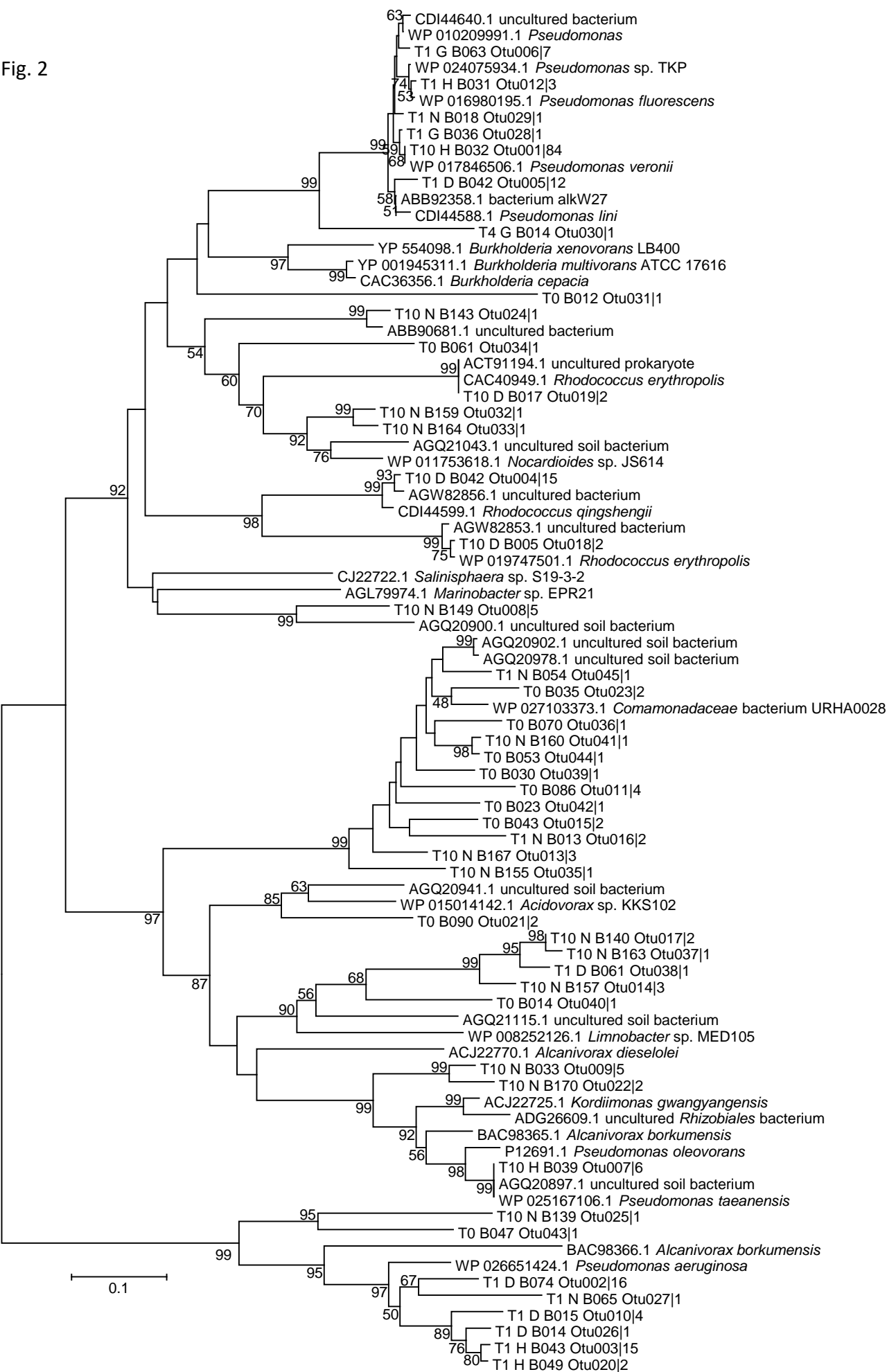
Figure 2. Phylogenetic distribution of *alkB* sequences. The phylogenetic tree was constructed from a ClustalW alignment of the derived amino acid sequences by neighbor-joining analysis with 1000 bootstrap replicates using Mega5.0 (Tamura et al. 2011). Bootstrap values greater than 50 are indicated.

Figure 3. P450 tree
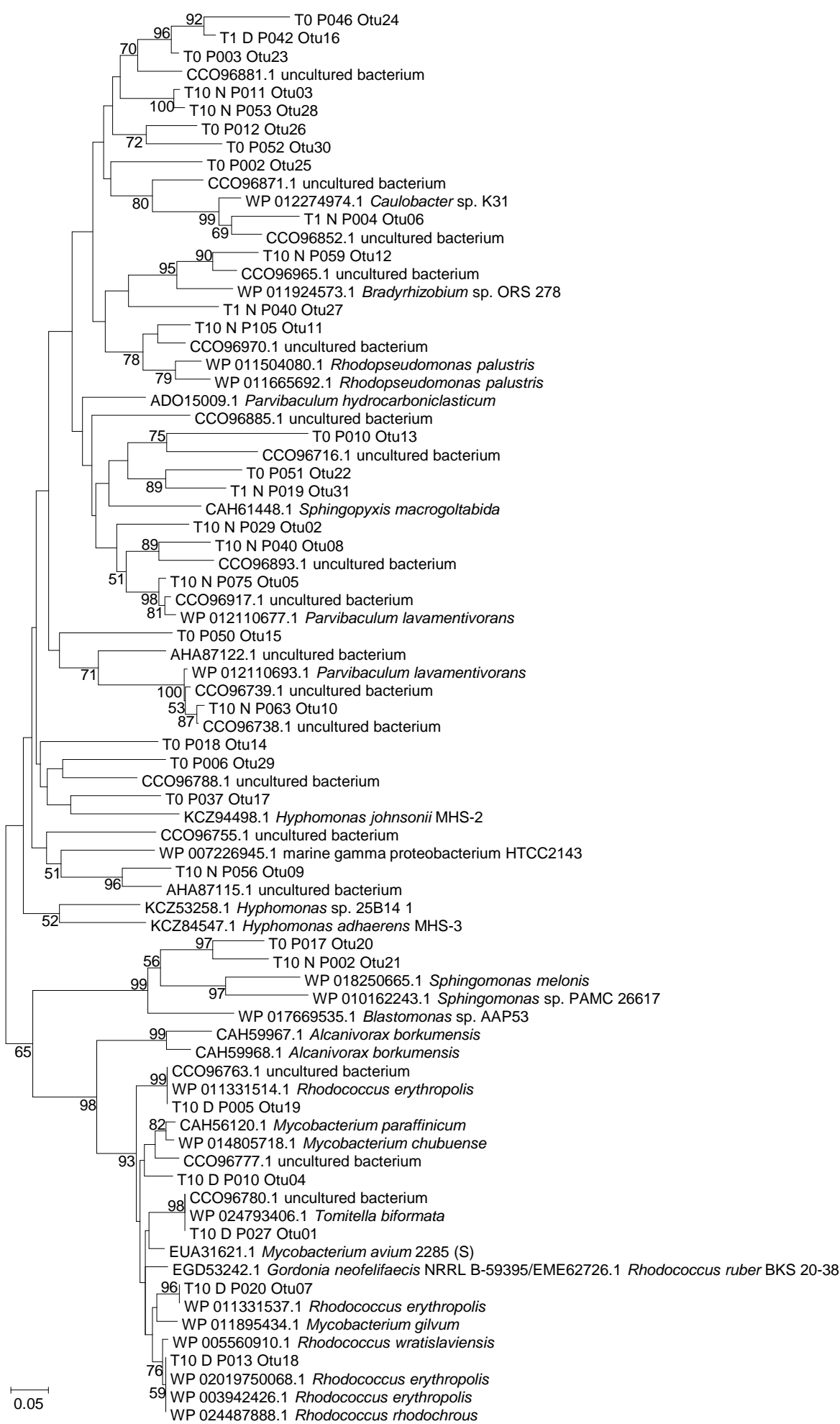
Figure 3. Phylogenetic distribution of *P450* sequences. The phylogenetic tree was constructed from a ClustalW alignment of the derived amino acid sequences by neighbor-joining analysis with 1000 bootstrap replicates using Mega5.0 (Tamura et al. 2011). Bootstrap values greater than 50 are indicated.
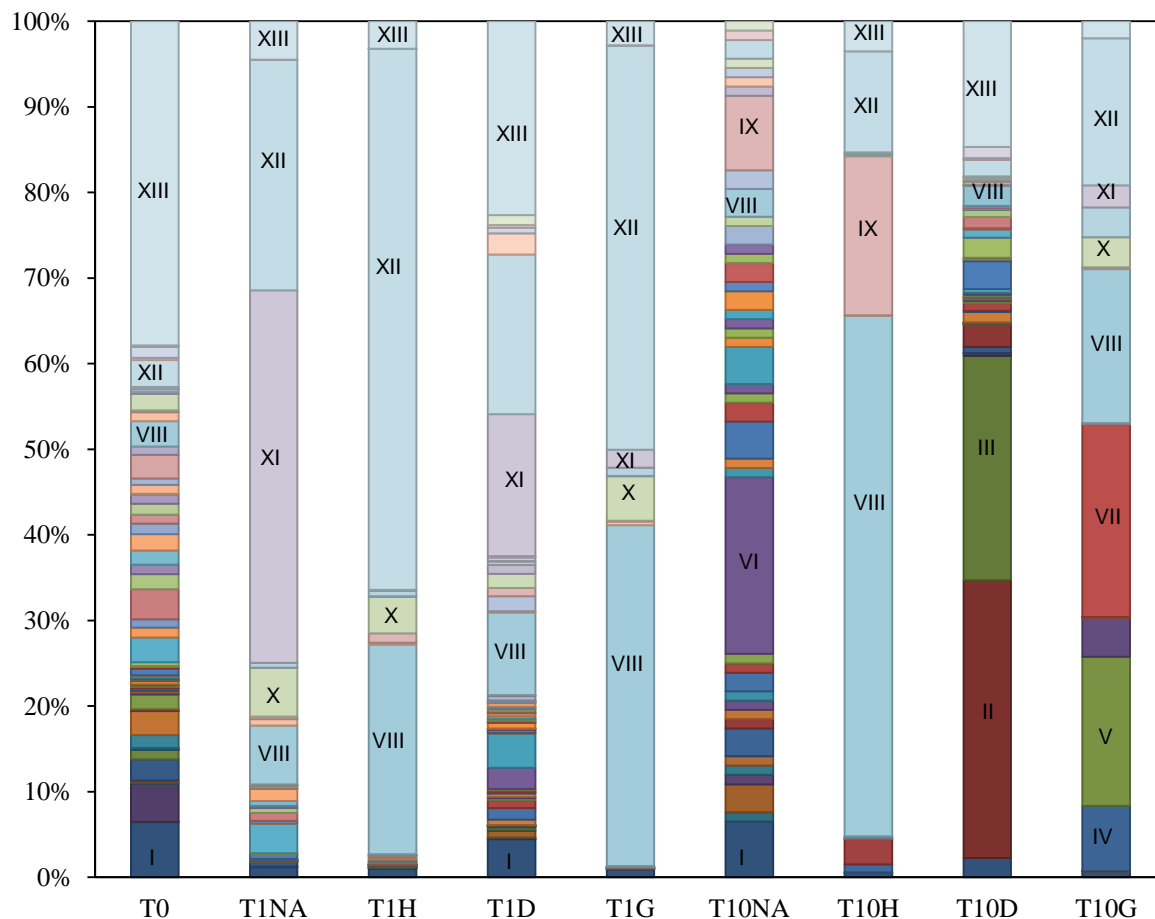
Figure 4. Bar graph showing distribution of tags into OTUs at the genus level. OTUs accounting for greater than 5% of an enrichment are labeled as follows: I, Other; II, Genus *Mycobacterium*; III, Genus *Rhodococcus*; IV, Genus *Bacillus*; V, Genus *Lysinibacillus*; VI, Family Aurantimonadaceae; VII, Family Acetobacteraceae; VIII, Class Gammaproteobacteria; IX, Genus *Marinobacter*; X, Genus *Shewanella*; XI, Genus *Acinetobacter*; XII, Genus *Pseudomonas*; XIII, groups that account for <1% of any enrichment.
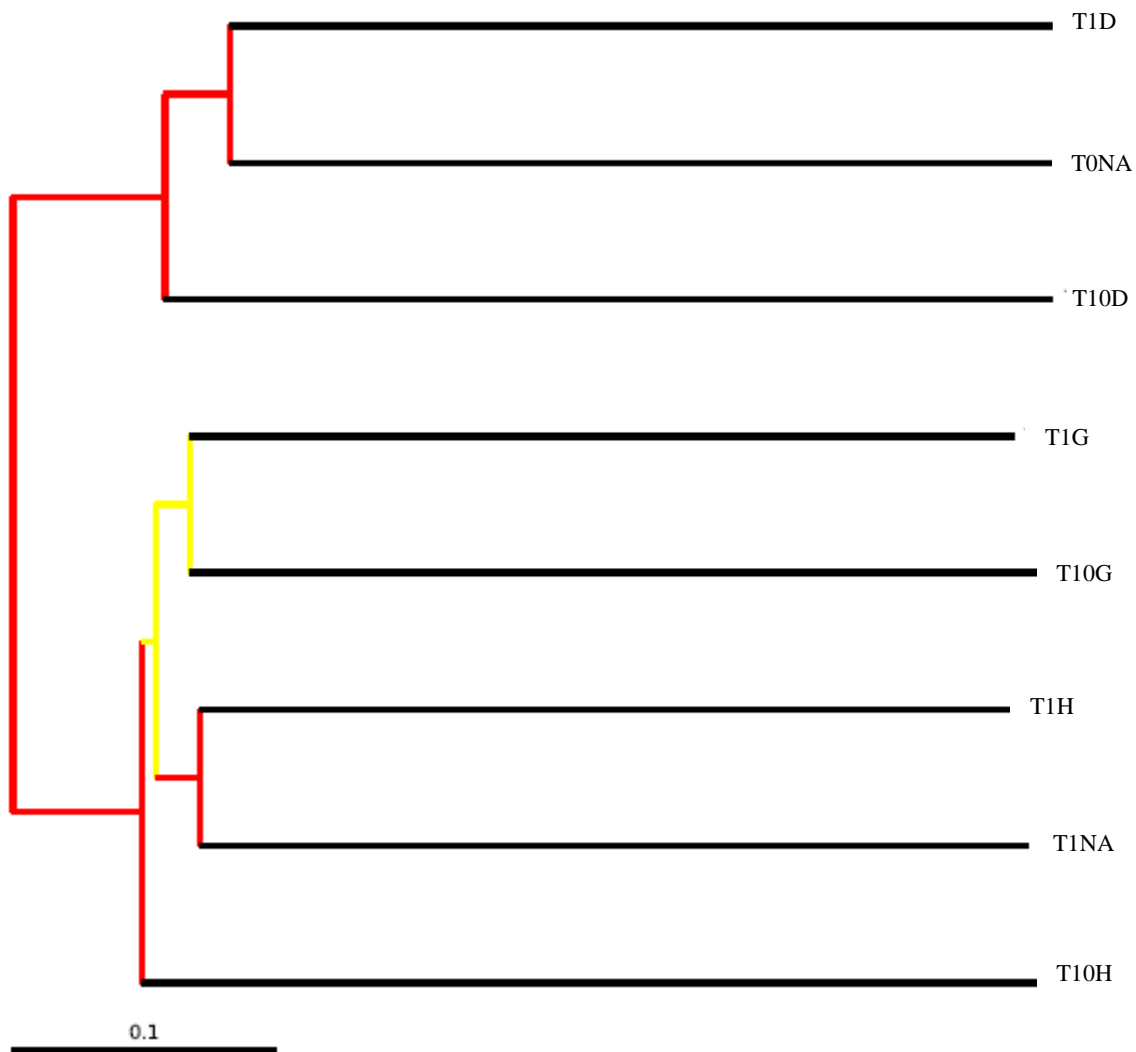
Figure 5. Dendrograms of hierarchical clustering of community similarities from all libraries (excluding the T10 NA due to low number of sequences recovered) based on weighted UniFrac Jackknife Cluster Analysis with 100 permutations (5000 sequence sub-sampling). Each node is colored by the fraction of times it was recovered in the jackknife replicates. Nodes recovered >99.9% of the time are red, 90–99.9% are yellow. T0, T1 and T10 indicate zero (initial), 1 day and 10 day enrichment samples. Alkanes are indicated as D (dodecane), H (hexane), G (gasoline) or NA (no alkane control).
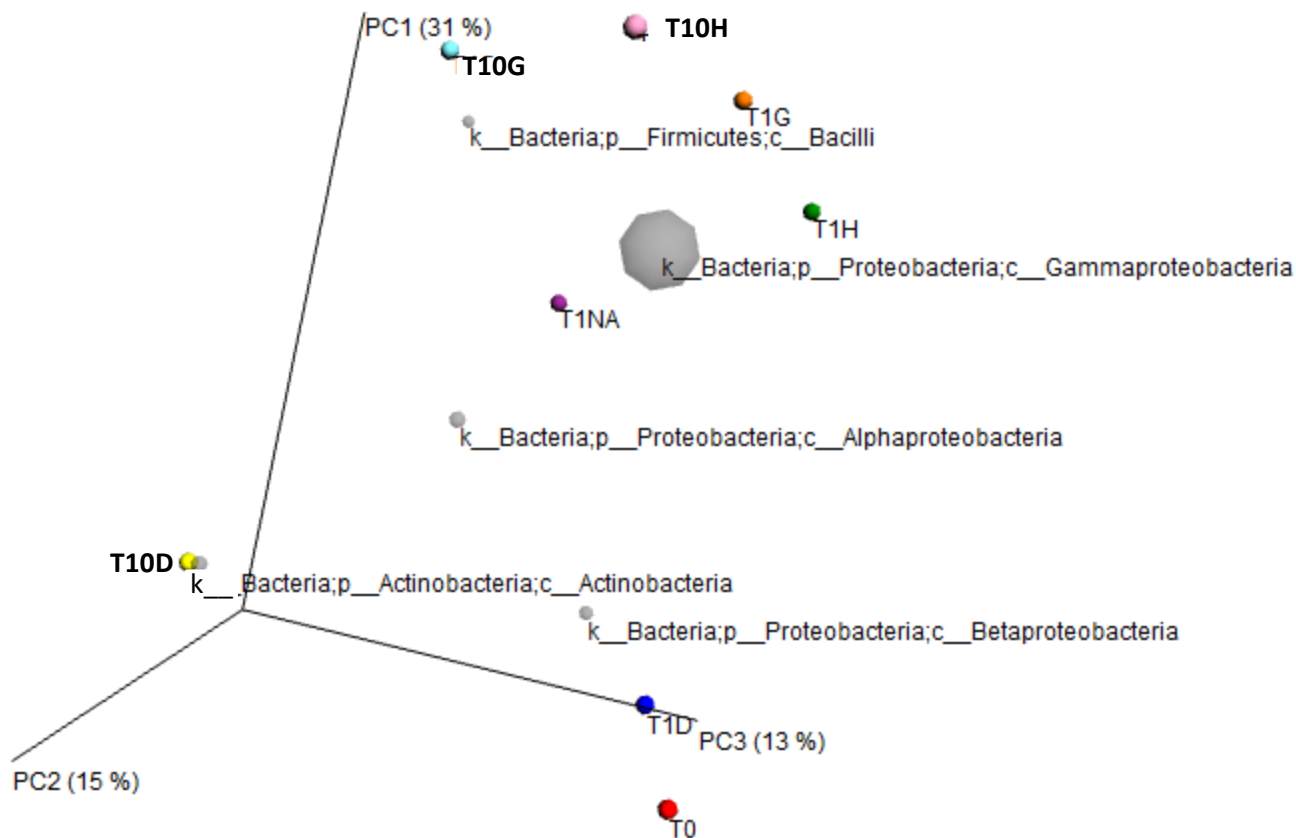
Figure 6. Principal coordinate analysis (PCoA) ordination based on an unweighted UniFrac distance matrix of sediment bacterial 16S rDNA gene profiles sampled from the initial sediment (T0), and after 1 and 10 days of exposure to dodecane (D), hexane (H) and gasoline (G) and no alkane control (NA). The T10 day no alkane control sample was not included due to the low number of sequences recovered. K ordination value labels indicate p = phylum and c = class.