Dear Editor,

Thank you for handling our manuscript.
Please find below our responses to the referee's comments. We have incorporated the suggestions of the referees into the final revised manuscript. All the changes made are highlighted in blue in the final revised manuscript.
Finally, we are grateful to the referees and you for considering our manuscript for publication in Biogeosciences.

Sincerely,
Zongze Shao, on behalf of all authors

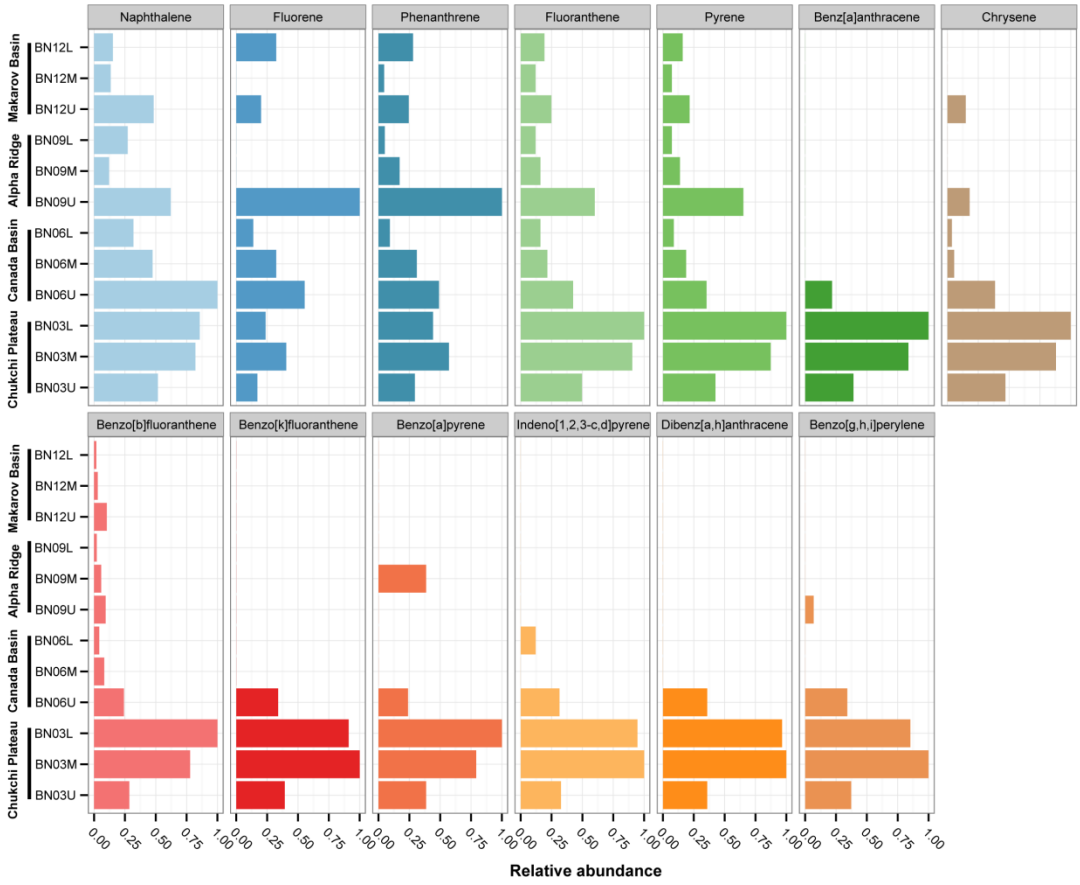
Response to Referee #1:

R.: This manuscript is basically in good shape. The authors have satisfactorily addressed my previous, minor reservations. I have a couple of technical suggestions which I strongly encourage the authors to adopt.

A.: Thanks. Your suggestions have been responded and followed as below.

R.: I must apologize to the authors: one of the suggestions arises from bad advice I gave in my previous review. The authors recreated Fig 3 using code I pointed them to. However, this code produced plots with unlabeled axes, which is unacceptable. I have included revised R code below to produce similar plots, but with labeled axes. I strongly encourage the authors to use the revised code (or to produce plots with labeled axes according to their own methods - it doesn't matter how precisely they do it, but it is essential to label plot axes!).

A.: Your corrections and further suggestions are deeply appreciated. We have recreated the figure your mentioned (Fig.2, not Fig.3 here) according to your revised R code.



R.: I also encourage the authors to rebuild Fig 3 using the same code. As I mentioned previously, stacked bar graphs are commonly used in microbial ecology, but I find them to be harder to interpret than the kind of plot in Fig 2. If these plots make sense for Fig 2, they probably make sense for Fig 3 as well.

A.: Thanks for your suggestion. However, we think there are no necessary to rebuild Fig. 3. Because most of dominant degrading bacteria have been presented in each sub-plots and the

current version of Fig.3 will be convenient to readers to make a comparison with previously studies.

In addition, there are more than 25 taxonomic units in each sub-plot of the Fig.3. Thus, the rebuilt figure will be very large if all the sub-plots were recreated according the revises R code.

R.: Lines 184-190 and Fig 4: I appreciate the authors including the equation for their calculation, but I still think that anything described as a "rate" needs to have units of time in the denominator. What the authors have calculated is more accurately described as "removal extent". It will be difficult to compare these data with any other experiment without dividing by the incubation time, which would give a true removal rate in units of %/hr. (If, for some reason, the authors really do not want to divide by incubation time, they should refer to the parameter they have calculated as "removal extent" and keep the units as %.)

A.: We have accepted this suggestion and changed “removal rate” to “removal extent” in the final revised manuscript and Fig.4.

Response to Referee #3:

A.: We thank the reviewer for accepting our paper.

Distribution of PAHs and the PAH-degrading bacteria in the deep-sea sediments of the high-latitude Arctic Ocean

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are common organic pollutants that can be transferred long distances and tend to accumulate in marine sediments. However, less is known regarding the distribution of PAHs and their natural bioattenuation in the open sea, especially the Arctic Ocean. In this report, sediment samples were collected at four sites from the Chukchi Plateau to the Makarov Basin in the summer of 2010. PAH compositions and total concentrations were examined with GC-MS. The concentrations of 16 EPA-priority PAHs varied from 2.0 to 41.6 ng g^{-1} dry weight and decreased with sediment depth and movement from the southern to the northern sites. Among the targeted PAHs, phenanthrene was relatively abundant in all sediments. The 16S rRNA gene of the total environmental DNA was analyzed with Illumina high throughput sequencing (IHTS) to determine the diversity of bacteria involved in PAH degradation *in situ*. The potential degraders including *Cycloclasticus*, *Pseudomonas*, *Halomonas*, *Pseudoalteromonas*, *Marinomonas*, *Bacillus*, *Dietzia*, *Colwellia*, *Acinetobacter*, *Alcanivorax*, *Salinisphaera* and *Shewanella*, with *Dietzia* as the most abundant, occurred in all sediment samples. Meanwhile, enrichment with PAHs was initiated on board and transferred to the laboratory for further enrichment and obtaining the degrading consortia. Most of the above mentioned bacteria in addition to *Hahella*, *Oleispira*, *Oceanobacter* and *Hyphomonas* occurred alternately as predominant members in the enrichment cultures from different sediments based on IHTS and PCR-DGGE analysis. To reconfirm their role in PAH degradation, 40 different bacteria were isolated and characterized, among which *Cycloclasticus* and *Pseudomonas* showed the best degradation capability under low temperatures. Taken together, PAHs and PAH-degrading bacteria were widespread in the deep-sea sediments of the Arctic Ocean. We propose that bacteria of *Cycloclasticus*, *Pseudomonas*, *Pseudoalteromonas*, *Halomonas*,

Marinomonas and *Dietzia* may play the most important role in PAHs mineralization *in situ*.

Keywords: polycyclic aromatic hydrocarbons (PAHs), biodegradation, bacterial diversity, deep-sea sediments, Arctic Ocean.

1 Introduction

The Arctic Ocean is the smallest major ocean and is almost completely surrounded by land. It also has the most extensive shelves of any ocean basin. The loading of terriginous organic carbon via rivers flowing into the Arctic Ocean (Opsahl et al., 1999;Lobbes et al., 2000;Benner et al., 2004) and the influence of terriginous organic matter on bacterial diversity in coastal waters have drawn attention (Ortega-Retuerta et al., 2012;Boeuf et al., 2014).

Polycyclic aromatic hydrocarbons (PAHs) are a type of aromatic hydrocarbons with two or more fused benzene rings. PAHs tend to accumulate in marine sediments, and are a source of significant environmental concern due to their toxicity, mutagenicity and carcinogenicity (Haritash and Kaushik, 2009). Because PAHs are one of the main components of crude oil (in addition to aliphatic hydrocarbons), the presence of PAHs in marine environments is mainly attributed to oil spills, discharge and natural seepage, river import, or even air current transfer (Latimer and Zheng, 2003). Therefore, the increase in human activities globally has increased the risks to marine environments.

The Arctic Ocean remains less exploited due to its remoteness and ice cover. However, worries concerning the Arctic ecosystem have increased in recent years. According to an assessment by the U.S. Geological Survey, approximately 30% and 13% of the world's undiscovered gas and oil reserves, respectively, may be found in the Arctic region (Gautier et al., 2009). Oil-drilling platforms have been set up on some offshore shelves (McClintock, 2011;Schmidt, 2012). With the oil exploitation and the future opening of the Northeast and Northwest passages, the increased input of PAHs into this area is unavoidable.

PAHs are widespread in marine coastal sediments (Baumard et al., 1998;Witt, 1995). They have also been found in surface sediments of the Arctic Ocean, with variable concentrations from

the shelf to basin (Yunker and Macdonald, 1995;Zaborska et al., 2011;Yunker et al., 2011). On the Beaufort Sea shelf, the total concentrations of PAHs with a molecular weight of 178-278 reached 850±230 ng g^{-1} dry weight (dw), with phenanthrene, benzo(*ghi*)perylene and benzo[*b*+*k*]fluoranthene as the dominant constituents (Yunker and Macdonald, 1995). In the western Barents Sea, the values of ΣPAHs ranged from 35 to 132 ng g^{-1} dw. Benzo[*b*+*k*]fluoranthene and phenanthrene dominated in the southern and northern areas, respectively (Zaborska et al., 2011). In contrast, the total concentration of PAHs was much lower in the high-latitude deep-sea basins, such as the Makarov Basin (35 ng g^{-1} dw) (Yunker et al., 2011).

As to the origin of PAHs in deep-sea sediments, long-range atmospheric transport and abiogenic production in deep-sea hydrothermal vents are believed to contribute to the accumulation of PAHs (Friedman and Selin, 2012;Proskurowski et al., 2008;Simoneit et al., 2004;Konn et al., 2009). In the case of the Arctic Ocean, it receives a large input of terrigenous and fossil particulate organic matters delivered by fluvial transport and coastal erosion in addition to combustion particulates contributed by atmospheric transport (Yunker et al., 2011). For example, industry in the former Soviet Union provided a widespread source of atmospheric PAHs to the Canadian High Arctic, which substantially decreased in the 1990s (Halsall et al., 1997;Becker et al., 2006). Therefore, these allochthonous PAHs could serve as carbon sources for various PAH-degrading bacteria in the Arctic sediments.

It is well known that bacterial degradation plays an important role in PAH removal from marine environments. Many PAH-degrading bacteria have been found in coastal sediments, including bacteria of *Cycloclasticus* (Dyksterhouse et al., 1995), *Marinobacter* (Hedlund et al., 2001), *Pseudoalteromonas* (Melcher et al., 2002), *Marinomonas* (Melcher et al., 2002),

Halomonas (Melcher et al., 2002), *Sphingomonas* (Demaneche et al., 2004), and *Vibrio* (Hedlund and Staley, 2001). However, less is known regarding deep-sea environments. In previous studies on the deep-sea sediments of the Atlantic Ocean and Pacific Ocean, we found that *Cycloclasticus* was the most important bacterium, in addition to *Alteromonas* and *Novosphingobium* (Cui et al., 2008;Shao et al., 2010;Wang et al., 2008). In the Arctic Ocean, *Pseudoalteromonas*, *Pseudomonas*, *Psychrobacter*, *Marinobacter* and *Shewanella* have been frequently reported as crude oil degraders in coastal seawater and sea ice (Deppe et al., 2005;Gerdes et al., 2005;Brakstad and Bonaunet, 2006;Giudice et al., 2010). *Pseudomonas* was found to be the predominant PAH degrader in terrestrial soils (Whyte et al., 1997;Sorensen et al., 2010;Eriksson et al., 2003). To the best of our knowledge, the diversity of PAH-degrading bacteria remains unknown in the deep-sea sediments of the high-latitude Arctic Ocean.

During the ecological survey of the "*Xuelong*" icebreaker in 2010, we sampled deep-sea sediments across the ocean and chose four sites at the Chukchi Plateau, Canada Basin, Alpha Ridge, and Makarov Basin to examine the distribution of PAHs and PAH-degrading bacteria therein. Bacterial diversity in both sediments and PAHs enrichment cultures were analyzed. The role of bacteria involved in PAH degradation was evaluated. The results will contribute to the depiction of the distribution pattern of PAHs and PAH-utilizing bacteria in this extreme environment, and help to evaluate the fate of PAHs following the contamination of such environments.

2 Material and Methods

2.1 Sediment collection

A total of 19 sediment cores were collected from the Chukchi Plateau, Canada Basin, Alpha

Ridge, and Makarov Basin during the fourth Arctic Research Expedition of the "*Xuelong*" icebreaker in the summer of 2010. PAHs and PAH-degrading bacteria are supposed to be varied in the regions with large distances. Based on this hypothesis, four sites representing the four typical geographical regions were chosen, including sites BN03, BN06, BN09 and BN12 (Fig. 1, Table S1). The sediment cores were first sampled using a box sampler (50×50×65 cm), then subsampled using a push core sampler (Φ10×60 cm) prior to releasing the box corers on deck. The length of the BN03, BN06, BN09, and BN12 cores were 20, 30, 24 and 38 centimeters below the surface, respectively. Subsequently, the cores were sliced into layers at depth intervals of 4 cm with the exception of the surface layer, which was sliced at a depth of 2 or 4 cm depending on the water content. Finally, three layers from each core, including the surface, the bottom and the middle (Table S1), were selected for analysis in this report. Approximately 5 g of sediment from each selected layer was used for PAH enrichment on board. The remains of the sediments were frozen immediately at -20 ℃ on board, transported to the home laboratory on dry ice, and stored at -80 ℃ until further analyses of PAH content and microbial diversity.

2.2 Chemicals and media

Naphthalene (>99.8%) was purchased from Sinopharm Chemical Reagent (Shanghai, China), and phenanthrene (>97%) and pyrene (>98%) were purchased from Sigma-Aldrich (St. Louis, USA). The Sixteen EPA priority pollutant PAH standards, six types of surrogate standards (1,4-dichlorobenzene-d4, naphthane-d8, acenaphthene-d10, chrysene-d12, phenanthrene-d10, and perylene-d12) and an internal standard (m-terphenyl) were purchased from AccuStandard (New Haven, USA). Surrogate and internal standards were used for quantifying procedural recovery and target PAH quantification, respectively. Crude oil was obtained from Iraq and imported by the SinoChem Quanzhou Petrochemical Corporation (Quanzhou, China). Mineral

medium, used for enrichment of PAH-degrading bacteria, contained 1 g of NH_4NO_3, 0.8 g of KH_2PO_4, 0.2 g of K_2HPO_4, 2.8×10-3 g of $FeSO_4$ and 1 liter of *in situ* deep-sea water from the Arctic Ocean. ONR7a medium, used for the cultivation of bacteria from the enriched cultures, contained 22.8 g of NaCl, 11.2 g of $MgCl_2•6H_2O$, 3.9 g of Na_2SO_4, 1.5 g of $CaC1_2•2H_2O$, 1.3 g of TAPSO, 0.7 g of KCl, 0.3 g of NH_4Cl, 89 mg of $Na_2HPO_4•7H_2O$, 83 mg of NaBr, 31 mg of $NaHCO_3$, 27 mg of H_3BO_3, 24 mg of $SrCl_2•6H_2O$, 2.6 mg of NaF, 2.0 mg of $FeCl_2•4H_2O$ and 1 liter of deionized water (Dyksterhouse et al., 1995).

2.3 PAHs quantification of deep-sea sediments

Extraction, purification, and gas chromatography-mass spectrometry (GC-MS) quantification of the PAHs in the deep-sea sediment samples were performed according to EPA method 8270D-2007 and previous reports (Zheng et al., 2002), with some modifications. Briefly, 0.05 mL of the 1 mg L^{-1} surrogate standard mixture solution was spiked into 20 g of freeze-dried sediment prior to extraction. Then, the sediment was placed into Extraction System B-811 (Buchi) and extracted with 250 ml of solvent consisting of a mixture of *n*-hexane and dichloromethane (1:1 v/v) under the hot extraction mode for 4 h. The extract was concentrated using a vacuum rotary evaporator and cleaned using column chromatography. The cleaned-up extract was further concentrated to 1 mL under a gentle N_2 stream. Finally, 0.05 mL of 1 mg L^{-1} m-terphenyl was added to the extract as an internal standard immediately before analysis.

All of the samples were analyzed using GC-MS run in the selected ion monitoring (SIM) mode. The molecular ion of each PAH was used for SIM. Sixteen target PAHs were identified based on both retention time relative to known standards and the mass of the molecular ion. Five calibration standard solutions (0.01-500 mg L^{-1}) containing the PAH standard, internal standard and surrogate compounds were carefully prepared, and a calibration curve was generated. The

mean of the relative response factors (RRFs) for each target PAH and the surrogate compounds was calculated. The quantifications were performed using the internal standard method, and the concentrations of target PAHs were corrected for the recoveries. The recoveries for surrogate standards were 81.6-105.2%. The lowest detection limit for each type of target PAH ranged from 0.13 to 0.97 ng g^{-1} dw.

2.4 PAH-degrading bacteria enrichment

Approximately 5 g of sediment from each selected layer was added to 250 mL of mineral medium in a 500-mL sterile polypropylene bottle. The sediments were supplied with 1 mL of crude oil spiked with a PAHs mixture of naphthalene, phenanthrene, and pyrene at final concentrations of 0.02, 0.01, and 0.005 g l^{-1}, respectively. Enrichment was performed on board at 4℃ and kept in the dark without agitation for two months. Only slight bacterial growth was observed after the first round of enrichment at 4℃. Once back to the home laboratory, the enriched cultures were transferred with an inoculum of 5% to 100 ml of fresh mineral medium in a 250-mL flask with the PAH mixture (without crude oil) as the sole carbon and energy source; this process was repeated twice in the rotary shakers (150 rpm) at 25℃ and 15℃ every one and two months, respectively. Finally, 36 PAH-degrading enriched cultures were obtained from the three temperature treatments.

2.5 PAH-removal extent quantification by GC-MS

To determine the PAH-removal extent of each consortium, all of the consortia and uninoculated controls were incubated in a 250-mL flask, which was loaded with 100 ml of fresh mineral medium containing 5% inoculum and the above-mentioned PAH mixtures as the carbon source. After a 45-day incubation at 15℃ and 25℃, the residual PAHs were extracted with 100 mL of dichloromethane separated into three parts. The purification and concentration of these combined

extracts was accomplished according to the description in "2.3 PAHs quantification of deep-sea sediments". The residual PAHs were quantified using an external standard method. The recovery rate for each of PAH was calculated based on the quantity difference before and after enrichment, extraction and purification in the uninoculated controls. The PAH-removal extent was calculated according to the formula:

$$\text{Removal extent (\%)} = \frac{tPAH - \left({}^{rPAH}/_{R}\right)}{tPAH} \times 100\%$$

tPAH: total quantity of each type of PAH before enrichment; rPAH: residual quantity of each type of PAH after enrichment; R: recovery rate of each type of PAH.

2.6 IHTS and data analysis

Community DNA of three selected layers from each core and the enriched cultures was extracted using the PowerSoil DNA Isolation Kit (MoBio) according to the manufacturer's instructions. Amplification of the 16S rRNA gene V6 region was performed using the universal bacterial primers 967F (5'-CNACGCGAAGAACCTTANC-3') and 1046R (5'-CGACAGCCATGCANCACCT-3') as previously described (Wu et al., 2010). A set of 10 nucleotide (nt) barcodes was designed and added to the 5' end of 967F for multiplexing of the samples in the Solexa paired-end (PE) sequencing runs. Each 25-μL PCR mixture consisted of approximately 10 ng of community DNA, 0.2 μM of each primer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 1× TaKaRa *Ex Taq* Buffer (Mg^{2+} free), and 2.5 units of TaKaRa *Ex Taq* DNA polymerase. PCR amplification was conducted using the following thermocycles: initial denaturation at 94 ℃ for 2 min; 25 cycles at 94 ℃ for 30 s, 57 ℃ for 30 s, and 72 ℃ for 30 s; and a final extension at 72 ℃ for 5 min. Equimolar amplicon suspensions were combined and subjected to paired-end sequencing using the PE75 strategy on the Illumina HiSeq2000 sequencing platform at the Beijing Berrygenomics Company.

206 **2.7 PCR-DGGE**

207 PCR amplification and DGGE analysis of the 16S rRNA gene V3 fragments of the enriched
208 consortia were performed as previously reported (Cui et al., 2008).

209 **2.8 Bacterial isolation, identification and phylogenetic analysis**

210 Cultures enriched at 15 ℃ were chosen as representatives for the isolation of degrading bacteria.
211 Approximately 10^{-4}, 10^{-5}, and 10^{-6} dilutions of theses cultures were spread onto ONR7a agar
212 plates whose lids were supplemented with naphthalene crystals and incubated at 15 ℃ in the dark.
213 Colonies with different morphologies were streaked onto fresh ONR7a plates twice to obtain
214 pure cultures. The PAH utilization of these isolates was tested in 100 mL of ONR7a liquid
215 medium supplemented with 0.2 g ultraviolet-sterilized naphthalene crystals at 15 ℃, reflected by
216 culture color changes and an increase in the cell optical density at 600 nm.

217 **2.9 Nucleotide sequence accession numbers**

218 The Illumina high-throughput sequencing data that resulted from the present study were
219 deposited into the NCBI Sequence Read Archive under run accession numbers
220 SRR975490-SRR975525 and SRR768499-SRR768507. The partial sequences of the 16S rRNA
221 gene obtained in this study were deposited into GenBank under accession numbers
222 KF470969-KF471008 (strains) and KC581800-KC581881 (DGGE bands).

223

224 # 3 Results

225 **3.1 PAHs in sediments**

226 GC-MS quantification indicated that the total concentration of the sixteen targeted PAHs
227 (Σ_{16}PAH) ranged from 2.02 to 41.63 ng g^{-1} dw (Table S2) and was decreased in the sediments
228 from the southern to northern sites (Fig. 2, Table S2). Among the sites, the southernmost site at

Chukchi Plateau (BN03) ranked the highest, whereas the northernmost site at Makarov Basin (BN12) ranked the lowest. At each site, the Σ_{16}PAH decreased with sediment depth, with the exception of the site at the Chukchi Plateau (BN03). The concentration of phenanthrene ranked the highest among the detected PAHs, followed by naphthalene; the only exception was the sediments of the Chukchi Plateau (Fig. 2, Table S2). PAHs with four to six rings, such as the four-ringed pyrene, five-ringed benzo[*b*]fluoranthene, and six-ringed benzo(*ghi*)perylene and indenopyrene, were significantly higher at the Chukchi Plateau site (BN03) compared to the other samples. At the Alpha Ridge site (BN09), phenanthrene ranked the highest (14.61 ng g^{-1} dw) among the PAHs of all samples.

3.2 Community structures of the *in situ* sediments revealed by IHTS

To obtain the bacterial composition and increased insights into the PAH degraders present in the *in situ* sediments, all 12 samples were subjected to Illumina high-throughput sequencing. The sequencing efforts and bacterial diversity indices are presented in the Supplemental Material in Table S3. Finally, a total of 1,152,388 raw reads were obtained, of which 1,051,978 clean reads were used for further analyses using QIIME (v 1.7.0).

At the phylum level, Proteobacteria were the most abundant bacteria and occupied 44.7%-57.3% of the total tags of these sediments, followed by Acidobacteria, Actinobacteria, Gemmatimonadetes, and Planctomycetes (Fig. S1A). The abundant orders are presented in the Supplemental Material (Fig. S1B). The top ten dominant bacteria belonged to *Oceanospirillales*, *Actinomycetales*, *Rhodospirillales*, *Planctomycetales*, *Gemmatimonadales*, *Acidobacteriales*, *Chromatiales*, *Alteromonadales*, *Pseudomonadales*, and *Bacillales*. Among them, *Oceanospirillales*, *Alteromonadales*, and *Pseudomonadales* contained most of known oil and PAH-degrading bacteria by far, such as *Pseudomonas*, *Cycloclasticus*, *Alcanivorax*,

Pseudoalteromonas and *Marinomonas.* More details are documented below.

The bacteria at the genus level are shown in Fig. 3A. The abundant bacteria that occupied more than 1% of the total tags in at least one sample are presented, including 20 known genera and 3 uncultured bacterial groups (Table S4). Among them, *Dietzia, Salinisphaera, Pseudomonas, Acinetobacter, Pseudoalteromonas, Colwellia, Bacillus, Rhodovibrio, Marinomonas,* and *Halomonas* have been reported as hydrocarbon-degrading bacteria in marine environments. In addition, *Cycloclasticus* and *Alcanivorax* are noteworthy because they have been recognized as obligate marine hydrocarbon degraders (Yakimov et al., 2007), and they were widespread in all of the sediments tested in this report (Table S4). Specially, *Cycloclasticus* occupied 0.2-0.5% of the 16S rRNA gene tags in each sample.

3.3 PAH degradation of the PAH enrichment consortia

All treatments displayed obvious bacterial growth following incubation under different temperatures (4 ℃, 15 ℃, and 25 ℃), reflected by color changes and changes in the turbidity of the cultures compared to the uninoculated controls. The PAH-removal extents of the consortia after 45 days of incubation at 15 ℃ and 25 ℃ were calculated based on the PAH concentrations determined by GC-MS. In general, the removal extents of phenanthrene and pyrene at 25 ℃ were higher than those at 15 ℃ (Fig. 4). Notably, the consortia of the northernmost site (BN12) generally displayed relatively high removal extents at 15 ℃ and 25 ℃. In contrast, the removal extents of the consortia from sites BN03, BN06, and BN09 were relatively low.

3.4 Community structures of the consortia enriched with PAHs

Thirty-six enrichment cultures corresponding to the *in situ* sediments were also analyzed using Illumina high-throughput sequencing (Table S3). Nearly all of the dominant bacteria in these consortia were previously described as hydrocarbon degraders. For the 4 ℃ treatments enriched

with crude oil-containing PAHs, the predominant bacteria included *Pseudomonas*, *Pseudoalteromonas*, *Marinomonas*, *Hahella*, *Marinobacter*, *Hyphomonas*, *Cycloclasticus*, *Colwellia*, *Halomonas*, *Oceanobacter*, *Salinisphaera*, *Oleispira*, *Alteromonas*, and *Alcanivorax* (Fig. 3B, Table S5). In the treatments that were enriched with PAHs at 15 ℃ and 25 ℃, *Pseudoalteromonas*, *Cycloclasticus*, *Pseudomonas*, and *Halomonas* were selected as the most abundant bacteria. In the 15 ℃ consortia (Fig. 3C), *Pseudoalteromonas* was the most abundant bacteria in the consortia of site BN03 and from the upper layer of BN06. In contrast, *Cycloclasticus* dominated the three consortia of site BN12 (55.1-64.5%), whereas *Pseudomonas* was clearly dominant in the consortia of the middle layer of site BN06 and upper layer of site BN09, and was relatively dominant in all three consortia from site BN12 (Fig. 3C, Table S5).

Halomonas was the most dominant bacterium in the 25 ℃ consortia (Fig. 3D), occupying 33.4-71.0% of the tags of the communities from sites BN03 and BN06, with the exception of the upper layer of BN03. *Pseudomonas* was dominant in the consortia of the middle layer of site BN09 (named 25BN09M, 54.1% of all tags), the lower layer of BN03 (25BN03L, 32.3%), the lower layer of BN06 (25BN06L, 31.6%), and the upper layer of BN12 (25BN12U, 21.6%). Similar to the 15 ℃ consortia, *Cycloclasticus* dominated the northernmost consortia at 25 ℃, such as the middle-layer consortium 25BN12M (46.0%), the lower-layer consortium 25BN12L (30.5%), and the upper-layer consortium 25BN12U (29.3%). Additionally, they were also relatively abundant in the consortium of the upper layer of the BN09 site (25BN09U, 9.1%).

3.5 Bacterial diversity comparisons based on IHTS data statistical analysis and environmental parameters

To compare the diversity indices, the tags were normalized to 7,047 (the fewest of the samples), and the observed OTUs, Chao1, and Shannon indices were obtained using the software package

QIIME (Table S3). Overall, all of the diversity indices indicated that the sediments had the highest bacterial richness and evenness (Fig. S2A-2C). Among the enriched cultures, the bacterial diversity was increased with rising temperatures (Fig. S2A-2B). The Chao1 values of the 25℃-enriched cultures were significantly higher compared to the other cultures ($P<0.01$); correspondingly, the observed OTU numbers of the 25℃-enriched cultures were also significantly higher than the 4℃ treatments ($P=0.029$). Principal coordinate analysis (PCoA) showed that the communities of the *in situ* sediments and all enriched cultures could be separated using the abundant OTU dataset (Fig. S2D), indicating that they had significantly different bacterial community structures; this finding was supported by the results of the nonparametric statistical Adonis method ($R^2=0.28$, $P=0.001$). Additionally, the Mantel test results showed no correlations between the community structures of the four types of samples and the individual or total PAH concentrations of the *in situ* sediments.

3.6 Community composition revealed by PCR-DGGE

To reconfirm the bacterial composition, the PAH-degrading consortia were subjected to PCR-DGGE analysis in parallel. Figure 5 presents the DGGE profiles of 12 consortia that were enriched with PAHs at 25℃. The bacteria, represented by bands, are listed in the Supplemental Material Table S6, and some are noted in the pattern profiles. In general, the community structures corresponded well to the IHTS results, even though the two methods targeted different regions of the 16S gene. In these consortia, the three genera *Cycloclasticus*, *Pseudomonas*, and *Halomonas* alternatively dominated the communities. Specifically, *Cycloclasticus* dominated all three consortia from site BN12 and the consortium (25BN09U) from the upper layer of site BN09; interestingly, it was accompanied by *Alcanivorax* (Fig. 5, lanes 7 and 10-12). This finding was in agreement with the results of the IHTS data (Fig. 3D). *Pseudomonas* dominated or shared

dominance with *Cycloclasticus* or *Halomonas* in four consortia (Fig. 5, lanes 3, 6, 8 and 10) that were derived from each layer of the four sites, whereas they were relatively less abundant in three consortia (Fig. 5, lanes 2, 4 and 11) that were generated from three sites. *Halomonas* appeared as very strong bands in five consortia (Fig. 5, lanes 2-6) from sites BN03 and BN06. *Pseudoalteromonas* mainly dominated in four consortia (Fig. 5, lanes 1, 3, 5 and 6) from sites BN03 and BN06. Other bacteria, such as *Marinobacter, Alcanivorax*, *Marinobacterium*, *Colwellia*, *Thalassospira*, *Celeribacter,* and *Vibrio*, were occasionally found to be strongly or weakly scattered in some of the consortia.

At low temperatures, the PAH-degrading communities varied to some extent in comparison to the 25 ℃ consortia. For example, in the three consortia derived from site BN12, bacteria such as *Cycloclasticus* and *Thalassolituus* became the dominant members at 4 ℃ (Fig. 6A, lanes 11-13), while bacteria such as *Pseudomonas, Maritalea*, and *Thalassospira* were only dominant members in 4BN12U and 4BN12M (Fig. 6A, lanes 11-12). In contrast, the most dominant member was the bacteria of *Cycloclasticus* at 15 ℃ (Fig. 6B, lane 11-13), which was consistent with the composition pattern revealed in Fig. 3C.

3.7 Bacterial isolation and their potential in PAH degradation

Bacteria were isolated from all of the PAH-degrading consortia enriched at 15 ℃ using the ONR7a medium plates whose lids were supplied with naphthalene crystals as the sole carbon source. Forty isolates were obtained that were affiliated with 12 genera of γ- and α-proteobacteria and Actinobacteria (Fig. S3). Bacteria belonging to γ-proteobacteria were the predominant isolates, including *Pseudoalteromonas* (18 isolates), *Halomonas* (6 isolates), *Cycloclasticus* (3 isolates), *Pseudomonas* (3 isolates), *Marinobacter* (2 isolates), and *Shewanella* (2 isolates). The PAH utilization test showed that only the bacteria of *Cycloclasticus* and

Pseudomonas showed obvious growth after two weeks in ONR7a liquid medium supplemented with naphthalene at 15 ℃.

Both the IHTS and PCR-DGGE results demonstrated that *Cycloclasticus* was predominant in the PAH-degrading consortia of site BN12 (Fig. 3, Fig. 5 and Fig. 6). From these consortia, three strains were isolated and named after the consortium: 15BN12U-14 (simplified as U-14), 15BN12L-10 (L-10), and 15BN12L-11 (L-11). These organisms had identical 16S rRNA gene sequences (1497 bp) and shared 99.92% sequence similarity with the *C. pugetii* PS-1^T type strain. However, they varied in morphology (Fig. S4) and genome fingerprint patterns determined by Rep-PCR (Fig. S5). Strains L-10 and L-11 resembled each other in morphology and Rep-PCR profiles; therefore, only strain L-10 was chosen for further analyses. Growth tests were conducted at 15 ℃ with a single PAH as the sole carbon source in ONR7a liquid medium. The results showed that strains L-10 and U-14 could assimilate naphthalene and phenanthrene, but neither could utilize pyrene (Fig. 7). Moreover, strain U-14 exhibited better growth than strain L-10 when utilizing phenanthrene (Fig. 7A *vs.* 7B).

4 Discussion

This report examined PAHs and PAH-degrading bacteria in the deep-sea sediments across the Arctic Ocean. Based on the data available to date, the total PAH concentrations decreased moving north toward the pole and generally decreased with sediment depth. Phenanthrene (0.64-14.61 ng g^{-1}) was the most dominant among the sixteen targeted EPA-priority PAHs. Based on the bacterial diversity data obtained using both culture-dependent and -independent methods, the general features of PAH-degrading bacteria were revealed in the sediment samples. To the best of our knowledge, this report is the first to consider the diversity and abundance of

PAH-utilizing bacteria in the deep-sea sediments of the high-latitude Arctic Ocean.

Recently, Yunker et al. examined the distribution of PAHs, plant odd alkanes, hopanes, and steranes in the sediments based on a large dataset to trace the origin of organic matter in the deep-sea sediments of the Arctic Ocean (Yunker et al., 2011). They found that the central Arctic Ocean basins were compositionally distinct from the rivers and shelves. Moreover, PAH concentrations decreased from 100-755 ng g^{-1} dw in the coastal sea to 35 ng g^{-1} dw in the central basin (Yunker et al., 2011). Our results in this report show that the PAH concentrations are generally in agreement with this tendency (Fig. 2, Table S2). According to the theory of "Global distillation", it would be easier to transfer naphthalene than other PAHs of high molecular weight (HMW) over long distances (Goldberg, 1975;Friedman and Selin, 2012); however, the concentration of naphthalene was less than that of phenanthrene. This discrepancy might be partially due to its higher bioavailability and degradability. In contrast, HMW PAHs with four or more rings accumulated in larger concentrations in areas near the south of the continent, as described above (Fig. 2). Additionally, the establishment of PAH compositions can likely be attributed to bacterial mineralization. To investigate the relationship between PAH composition and bacterial communities, we analyzed the correlations between the community structures and the concentrations of individual or total PAHs using the Mantel test method. However, no definite correlation was observed. Bacterial communities *in situ* may be influenced by many other factors, such as nutrients and other carbon sources, in addition to the PAHs in the tested samples from the Arctic Ocean.

Various bacteria involved in PAH-degradation were identified in all of the sediments, including *Cycloclasticus*, *Pseudomonas*, *Halomonas*, *Pseudoalteromonas*, *Marinomonas*, *Bacillus*, *Dietzia*, *Colwellia*, *Acinetobacter*, *Alcanivorax*, *Salinisphaera* and *Shewanella*.

However, most of these bacteria occupied less than 0.5% of the total tags (Fig. S6). After PAH enrichment, *Pseudomonas*, *Pseudoalteromonas*, *Cycloclasticus*, *Halomonas*, and *Marinomonas* became the dominant members in the enriched cultures (Fig. S6). For example, when enriched at 4℃ with PAHs dissolved in crude oil, *Pseudomonas*, *Pseudoalteromonas*, *Marinomonas*, *Hyphomonas*, and *Cycloclasticus* were identified as the dominant members (Fig. 3B and Fig. S6). These bacteria have been previously detected as the dominant members in oil-enriched consortia of the coastal seawater and sea ice from the Arctic Ocean (Deppe et al., 2005;Gerdes et al., 2005;Brakstad and Bonaunet, 2006;Giudice et al., 2010). These findings are in contrast to those from the deep-sea oil plume that occurred during the Deepwater Horizon oil spill, which was dominated by bacteria of the order *Oceanospirillales* and the genus *Colwellia* (Hazen et al., 2010;Baelum et al., 2012). When enriched with PAHs as a sole carbon and energy source at 15℃ and 25℃, the obtained PAH-degrading consortia were alternately dominated by *Pseudomonas*, *Pseudoalteromonas*, *Halomnonas*, and *Cycloclasticus*. A big difference in the community structures occurred between cultures grown at 4℃ and 15℃ (Fig. 3 and Fig. S2); while the bacterial community structures grown at 25℃ also varied to some extent in comparison with those grown at 15℃, the dominant bacteria, including *Cycloclasticus*, *Pseudomonas*, *Pseudoalteromonas* and *Halomnonas*, remained predominant (Fig. 3). The alpha and beta diversity indices (Fig. S2) both confirmed that significant differences existed among the consortia enriched under different temperatures, and indicated that temperature substantially influenced the bacterial community structure. This could be explained by the enhancement of PAH availability and the metabolic activity of the bacteria at high temperatures.

Bacteria of the genus *Cycloclasticus* have been recognized as obligate marine PAH degraders (Dyksterhouse et al., 1995;Yakimov et al., 2007). They usually represent one of the most

predominant genera detected in crude oil-polluted sediments or seawater (Kasai et al., 2002;Maruyama et al., 2003;McKew et al., 2007a;McKew et al., 2007b;Coulon et al., 2007;Kappell et al., 2014;Dubinsky et al., 2013). In addition to coastal environments, they have also been found in the deep-sea sediments of both the Atlantic and Pacific Oceans, as described in our previous reports based on culture enrichment (Cui et al., 2008;Shao et al., 2010;Wang et al., 2008). This report is the first to use IHTS to confirm the wide distribution of *Cycloclasticus* bacteria in deep-sea sediments. In Arctic deep-sea sediments, bacteria of this genus occupied 0.2%-0.5% of the total bacteria sequenced in each sediment sample *in situ* based on the detection of sequence tags on the 16S rRNA gene. Therefore, it seems likely that they play an important role in PAH mineralization in this environment. Interestingly, the abundance of *Cycloclasticus* increased with sediment depth and movement from the southern to northern sites (Table S4); this finding was in contrast to PAH concentrations that decreased with depth and movement from the south to the north. This finding is most likely due to the fact that the labile carbon sources are relatively abundant in the surface sediments and are reduced in the older sediments (deep layers) and remote areas such as BN12, which is close to the North Pole. In the sediments where labile carbon sources are scarce, the PAHs may represent a key factor in the selection of PAH-degrading bacteria that adapt to the oligotrophic circumstances.

Cycloclasticus bacteria were found in all twelve of the *in situ* sediment samples, but were difficult to cultivate on plate, even when a simple carbon source, such as acetate and pyruvate, was used. After many attempts, three strains were finally obtained on the ONR7a medium plates supplied with naphthalene crystals on the lids, showing tiny colonies after three weeks of incubation at 15℃. Growth tests indicated that these *Cycloclasticus* strains could use naphthalene or phenanthrene as a sole carbon source, but failed to utilize pyrene. In the previous

436 study, we isolated a pyrene-degrading bacterium from a Pacific deep-sea sediment that
437 represented the only one strain of *Cycloclasticus* reported to date that is capable of using pyrene
438 as its sole carbon and energy source (Wang et al., 2008); in contrast, other strains can utilize
439 pyrene only in the presence of other PAHs, such as phenanthrene (Geiselbrecht et al., 1998).
440 In addition to *Cycloclasticus*, the following PAH-degrading bacteria, which were previously
441 described to reside in coastal environments, were also found as dominant members in some
442 PAH-degrading consortia in this report (see Supplemental Material Table S5): *Pseudomonas*
443 (Niepceron et al., 2010), *Marinomonas* (Melcher et al., 2002), *Pseudoalteromonas* (Hedlund and
444 Staley, 2006), *Halomonas* (Garcia et al., 2005), *Alteromonas* (Jin et al., 2012), *Marinobacter*
445 (Hedlund et al., 2001), *Vibrio* (Hedlund and Staley, 2001), and *Thalassospira* (Kodama et al.,
446 2008). In fact, we also isolated 64 strains from the consortia enriched at 25℃ using M2 media
447 plates (Wang et al., 2008), which contain more distinct carbon compounds than ONR7a media
448 plates (i.e., sodium acetate, glucose, sucrose, sodium citrate, and malic acid). Among them, only
449 three strains (*Marinomonas* sp. D104, *Sphingobium* sp. C100 and *Pseudomonas* sp. C39) showed
450 a good PAH-degradation capability at 15℃ and 25℃. Genome sequencing revealed that strains
451 D104 and C100 possessed several genes involved in the initial hydroxylation and intermediate
452 metabolic steps of PAHs (Dong et al., 2014b, a). Particularly, strain *Marinomonas* sp. D104
453 could even degrade the PAH mixture of naphthalene, phenanthrene, and pyrene at 4℃
454 (unpublished data). Although *Pseudoalteromonas* and *Halomonas* were the most predominant
455 members in many consortia (Fig. 3 and Fig. 5), in this study they failed to grow in the presence
456 of the tested PAHs.
457 *Pseudomonas* is a common PAH-degrader in cold environments, and is frequently found in
458 Arctic and Antarctic soils (Whyte et al., 1997;Sorensen et al., 2010;Eriksson et al., 2003;Ma et

al., 2006). In this study, *Pseudomonas* occurred *in situ* as one of the most dominant bacteria and occupied 1.5-1.8% of the total bacteria sequenced from the three samples (Table S4 and Fig. 3A). In many cultures enriched with PAHs, *Pseudomonas* was the dominant member and even occupied up to 70% of the total populations in the 4BN03M consortium (Table S5 and Fig. 3B). Coincidently, three *Pseudomonas* strains were obtained from the 15 ℃ enrichments (Fig. S3) and were able to grow with naphthalene. However, to the best of our knowledge, *Pseudomonas* is less abundant in oceanic sediments (Cui et al., 2008;Wang et al., 2008;Shao et al., 2010).

In addition to the bacteria mentioned above, many other bacteria belonging to *Dietzia*, *Alcanivorax*, *Colwellia*, *Thalassolituus*, *Oceanobacter, Hahella,* and *Roseovarius* were also relatively dominant in some of the PAH-degrading communities. *Dietzia* (Alonso-Gutierrez et al., 2011), *Alcanivorax* (Schneiker et al., 2006), *Colwellia* (Baelum et al., 2012), *Thalassolituus* (Yakimov et al., 2004) and *Oceanobacter* (Teramoto et al., 2009) have been reported as hydrocarbon-degrading bacteria, but not as PAH degraders. It is noteworthy that bacteria of *Dietzia* were abundant *in situ*, particularly in the middle or lower layers of all sediments, and occupied 1.65%-7.8% of the total tags (Fig. 3A, Table S4). These organisms might thrive on alkanes in these environments, but are not likely to thrive on PAHs because they only occupied 11.25% of the total tags in one consortium (the upper layer of site BN03, enriched at 15 ℃) (Fig. 3C, Table S5). Interestingly, bacteria of *Thalassolituus* occurred as the rare species (abundance <0.01%) in all *in situ* sediments. They became predominant in the 4 ℃ cultures of site BN12 enriched with oil containing PAHs (Fig. 6A), but were diluted from the PAH-enriched cultures at both 15 ℃ and 25 ℃ (Fig. 6B and Fig. 5). These results were quite consistent with their aliphatic hydrocarbon-degrading and psychrotolerant characteristics (Yakimov et al., 2004).

It is worth mentioning that the once frequently reported PAH-degrading bacteria

Novosphingobium spp. were not detected in the sediments of the Arctic Ocean. These bacteria are common PAH degraders in both marine and terrestrial environments (Gan et al., 2013). In our previous reports, they occurred as a predominant PAH degrader in nearly all PAH enrichment cultures, such as those from the deep-sea column of the Indian Ocean (Yuan et al., 2009) and those from hydrothermal sediments of the Lau Basin (Dong et al., 2011). Other reports also proved the widespread distribution of *Novosphingobium* bacteria and confirmed their roles as effective PAH degraders (Balkwill et al., 1997;Sohn et al., 2004;Yuan et al., 2009;Notomista et al., 2011). Similar to *Novosphingobium*, bacteria of *Sphingomonas* and *Neptunomonas* were not found in the sediments of the Arctic Ocean, although they are also common PAH-degraders in marine environments (Demaneche et al., 2004;Hedlund et al., 1999).

In summary, various PAHs and degrading bacteria are ubiquitous in the Arctic deep-sea sediments. In general, the total PAH concentrations decreased with sediment depths and movement from the south to the north, and ranged from 2.0 to 41.6 ng g^{-1} dw. Correspondingly, various bacteria involved in PAH degradation existed in the deep-sea sediments, including the obligate marine hydrocarbon-degrading bacteria *Cycloclasticus*, *Alcanivorax*, and *Thalassolituus*, as well as *Pseudomonas*, *Pseudoalteromonas*, *Marinobacter*, *Marinomonas*, *Acinetobacter*, *Bacillus*, *Colwellia*, *Dietzia*, *Halomonas*, *Rhodovibrio*, *Salinisphaera* and *Shewanella*. Among them, *Cycloclasticus*, *Pseudomonas*, *Pseudoalteromonas*, *Marinomonas*, *Halomonas* and *Dietzia* may play a more important role in PAH degradation *in situ* in the Arctic Ocean. Bioattenuation of PAHs occurs whilst bacteria survive in the remote deep-sea areas, which are cold, dark, oligotrophic, high pressure, and perennially covered in ice.

Supplementary material

The supplement related to this article is uploaded during the manuscript submission.

Author contribution. Z. Z. S. and C. M. D. designed research; X. H. B. and C. M. D. performed research; C. M. D., Z. Z. S., X. H. B., H. F. S., L. P. J. and H. W. Z. analyze data; C. M. D. and Z. Z. S. contributed to the writing of the paper.

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References

Alonso-Gutierrez, J., Teramoto, M., Yamazoe, A., Harayama, S., Figueras, A., and Novoa, B.: Alkane-degrading properties of *Dietzia* sp. H0B, a key player in the Prestige oil spill biodegradation (NW Spain), Journal of applied microbiology, 111, 800-810, 10.1111/j.1365-2672.2011.05104.x, 2011.

Baelum, J., Borglin, S., Chakraborty, R., Fortney, J. L., Lamendella, R., Mason, O. U., Auer, M., Zemla, M., Bill, M., Conrad, M. E., Malfatti, S. A., Tringe, S. G., Holman, H. Y., Hazen, T. C., and Jansson, J. K.: Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill, Environ Microbiol, 14, 2405-2416, 10.1111/j.1462-2920.2012.02780.x, 2012.

Balkwill, D. L., Drake, G. R., Reeves, R. H., Fredrickson, J. K., White, D. C., Ringelberg, D. B., Chandler, D. P., Romine, M. F., Kennedy, D. W., and Spadoni, C. M.: Taxonomic study of aromatic-degrading bacteria from deep-terrestrial-subsurface sediments and description of *Sphingomonas aromaticivorans* sp. nov., *Sphingomonas subterranea* sp. nov., and *Sphingomonas stygia* sp. nov, Int J Syst Bacteriol, 47, 191-201, 1997.

Baumard, P., Budzinski, H., and Garrigues, P.: Polycyclic aromatic hydrocarbons in sediments and mussels of the western Mediterranean sea, Environmental Toxicology and Chemistry, 17, 765-776, 10.1002/etc.5620170501, 1998.

Becker, S., Halsall, C. J., Tych, W., Hung, H., Attewell, S., Blanchard, P., Li, H., Fellin, P., Stern, G., Billeck, B., and Friesen, S.: Resolving the Long-Term Trends of Polycyclic Aromatic Hydrocarbons in the Canadian Arctic Atmosphere, Environmental science & technology, 40, 3217-3222, 10.1021/es052346l,

2006.
Benner, R., Benitez-Nelson, B., Kaiser, K., and Amon, R. M. W.: Export of young terrigenous dissolved organic carbon from rivers to the Arctic Ocean, Geophysical Research Letters, 31, L05305, 10.1029/2003GL019251, 2004.
Boeuf, D., Humily, F., and Jeanthon, C.: Diversity of Arctic pelagic Bacteria with an emphasis on photoheterotrophs: a review, Biogeosciences, 11, 3309-3322, 10.5194/bg-11-3309-2014, 2014.
Brakstad, O. G., and Bonaunet, K.: Biodegradation of petroleum hydrocarbons in seawater at low temperatures (0-5 degrees C) and bacterial communities associated with degradation, Biodegradation, 17, 71-82, 10.1007/s10532-005-3342-8, 2006.
Coulon, F., McKew, B. A., Osborn, A. M., McGenity, T. J., and Timmis, K. N.: Effects of temperature and biostimulation on oil-degrading microbial communities in temperate estuarine waters, Environ Microbiol, 9, 177-186, 10.1111/j.1462-2920.2006.01126.x, 2007.
Cui, Z., Lai, Q., Dong, C., and Shao, Z.: Biodiversity of polycyclic aromatic hydrocarbon-degrading bacteria from deep sea sediments of the Middle Atlantic Ridge, Environ Microbiol, 10, 2138-2149, 2008.
Demaneche, S., Meyer, C., Micoud, J., Louwagie, M., Willison, J. C., and Jouanneau, Y.: Identification and functional analysis of two aromatic-ring-hydroxylating dioxygenases from a *sphingomonas* strain that degrades various polycyclic aromatic hydrocarbons, Appl Environ Microbiol, 70, 6714-6725, 10.1128/AEM.70.11.6714-6725.2004, 2004.
Deppe, U., Richnow, H. H., Michaelis, W., and Antranikian, G.: Degradation of crude oil by an arctic microbial consortium, Extremophiles : life under extreme conditions, 9, 461-470, 10.1007/s00792-005-0463-2, 2005.
Dong, C., Chen, L., Liao, Y., and Shao, Z.: Phylogenetic and degrading genes analysis of a PAHdegrading bacterium TVG9-VII from deep-sea hydrythermal environment, Acta Microbiologica Sinica, 51, 1548-1554, 2011.
Dong, C., Bai, X., Lai, Q., Xie, Y., Chen, X., and Shao, Z.: Draft Genome Sequence of *Sphingobium* sp. Strain C100, a Polycyclic Aromatic Hydrocarbon-Degrading Bacterium from the Deep-Sea Sediment of the Arctic Ocean, Genome announcements, 2, 10.1128/genomeA.01210-13, 2014a.
Dong, C., Bai, X., Lai, Q., Xie, Y., Chen, X., and Shao, Z.: Draft Genome Sequence of *Marinomonas* sp. Strain D104, a Polycyclic Aromatic Hydrocarbon-Degrading Bacterium from the Deep-Sea Sediment of the Arctic Ocean, Genome announcements, 2, 10.1128/genomeA.01211-13, 2014b.
Dubinsky, E. A., Conrad, M. E., Chakraborty, R., Bill, M., Borglin, S. E., Hollibaugh, J. T., Mason, O. U., Y., M. P., Reid, F. C., Stringfellow, W. T., Tom, L. M., Hazen, T. C., and Andersen, G. L.: Succession of hydrocarbon-degrading bacteria in the aftermath of the deepwater horizon oil spill in the gulf of Mexico, Environmental science & technology, 47, 10860-10867, 10.1021/es401676y, 2013.
Dyksterhouse, S. E., Gray, J. P., Herwig, R. P., Lara, J. C., and Staley, J. T.: *Cycloclasticus pugetii* gen. nov., sp. nov., an aromatic hydrocarbon-degrading bacterium from marine sediments, Int J Syst Bacteriol, 45, 116-123, 1995.
Eriksson, M., Sodersten, E., Yu, Z., Dalhammar, G., and Mohn, W. W.: Degradation of Polycyclic Aromatic Hydrocarbons at Low Temperature under Aerobic and Nitrate-Reducing Conditions in Enrichment Cultures from Northern Soils, Appl Environ Microbiol, 69, 275-284, 2003.
Friedman, C. L., and Selin, N. E.: Long-range atmospheric transport of polycyclic aromatic hydrocarbons: a global 3-D model analysis including evaluation of Arctic sources, Environmental science & technology, 46, 9501-9510, 10.1021/es301904d, 2012.
Gan, H. M., Hudson, A. O., Rahman, A. Y., Chan, K. G., and Savka, M. A.: Comparative genomic analysis of six bacteria belonging to the genus *Novosphingobium*: insights into marine adaptation, cell-cell signaling and bioremediation, BMC genomics, 14, 431, 10.1186/1471-2164-14-431, 2013.
Garcia, M. T., Ventosa, A., and Mellado, E.: Catabolic versatility of aromatic compound-degrading halophilic bacteria, FEMS Microbiol Ecol, 54, 97-109, 10.1016/j.femsec.2005.03.009, 2005.
Gautier, D. L., Bird, K. J., Charpentier, R. R., Grantz, A., Houseknecht, D. W., Klett, T. R., Moore, T. E., Pitman, J. K., Schenk, C. J., and Schuenemeyer, J. H.: Assessment of undiscovered oil and gas in the Arctic, Science, 324, 1175-1179, 2009.

Geiselbrecht, A. D., Hedlund, B. P., Tichi, M. A., and Staley, J. T.: Isolation of Marine Polycyclic Aromatic Hydrocarbon (PAH)-Degrading *Cycloclasticus* Strains from the Gulf of Mexico and Comparison of Their PAH Degradation Ability with That of Puget Sound *Cycloclasticus* Strains, Applied and Environmental Microbiology, 64, 4703-4710, 1998.

Gerdes, B., Brinkmeyer, R., Dieckmann, G., and Helmke, E.: Influence of crude oil on changes of bacterial communities in Arctic sea-ice, FEMS Microbiology Ecology, 53, 129-139, 10.1016/j.femsec.2004.11.010, 2005.

Giudice, A. L., Bruni, V., Domenico, M. D., and Michaud, L.: Psychrophiles - Cold-Adapted Hydrocarbon-Degrading Microorganisms, in: Handbook of Hydrocarbon and Lipid Microbiology, edited by: Timmis, K. N., Springer Berlin Heidelberg, 1897-1921, 2010.

Goldberg, E. D.: Synthetic organohalides in the sea, Proc R Soc Lond B Biol Sci, 189, 277-289, 1975.

Halsall, C. J., Barrie, L. A., Fellin, P., Muir, D. C. G., Billeck, B. N., Lockhart, L., Rovinsky, F. Y., Kononov, E. Y., and Pastukhov, B.: Spatial and Temporal Variation of Polycyclic Aromatic Hydrocarbons in the Arctic Atmosphere, Environmental science & technology, 31, 3593-3599, 10.1021/es970342d, 1997.

Haritash, A. K., and Kaushik, C. P.: Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review, J Hazard Mater, 169, 1-15, 10.1016/j.jhazmat.2009.03.137, 2009.

Hazen, T. C., Dubinsky, E. A., DeSantis, T. Z., Andersen, G. L., Piceno, Y. M., Singh, N., Jansson, J. K., Probst, A., Borglin, S. E., Fortney, J. L., Stringfellow, W. T., Bill, M., Conrad, M. E., Tom, L. M., Chavarria, K. L., Alusi, T. R., Lamendella, R., Joyner, D. C., Spier, C., Baelum, J., Auer, M., Zemla, M. L., Chakraborty, R., Sonnenthal, E. L., D'Haeseleer, P., Holman, H. Y., Osman, S., Lu, Z., Van Nostrand, J. D., Deng, Y., Zhou, J., and Mason, O. U.: Deep-sea oil plume enriches indigenous oil-degrading bacteria, Science, 330, 204-208, 10.1126/science.1195979, 2010.

Hedlund, B. P., Geiselbrecht, A. D., Bair, T. J., and Staley, J. T.: Polycyclic aromatic hydrocarbon degradation by a new marine bacterium, *Neptunomonas naphthovorans* gen. nov., sp. nov, Appl Environ Microbiol, 65, 251-259, 1999.

Hedlund, B. P., Geiselbrecht, A. D., and Staley, J. T.: *Marinobacter* strain NCE312 has a Pseudomonas-like naphthalene dioxygenase, FEMS microbiology letters, 201, 47-51, 10.1111/j.1574-6968.2001.tb10731.x, 2001.

Hedlund, B. P., and Staley, J. T.: *Vibrio cyclotrophicus* sp. nov., a polycyclic aromatic hydrocarbon (PAH)-degrading marine bacterium, International journal of systematic and evolutionary microbiology, 51, 61-66, 2001.

Hedlund, B. P., and Staley, J. T.: Isolation and characterization of *Pseudoalteromonas* strains with divergent polycyclic aromatic hydrocarbon catabolic properties, Environmental Microbiology, 8, 178-182, 2006.

Jin, H. M., Kim, J. M., Lee, H. J., Madsen, E. L., and Jeon, C. O.: *Alteromonas* As a Key Agent of Polycyclic Aromatic Hydrocarbon Biodegradation in Crude Oil-Contaminated Coastal Sediment, Environmental science & technology, 46, 7731-7740, 10.1021/es3018545, 2012.

Kappell, A. D., Wei, Y., Newton, R. J., Van Nostrand, J. D., Zhou, J., McLellan, S. L., and Hristova, K. R.: The polycyclic aromatic hydrocarbon degradation potential of Gulf of Mexico native coastal microbial communities after the Deepwater Horizon oil spill, Front Microbiol, 5, 205, 10.3389/fmicb.2014.00205, 2014.

Kasai, Y., Kishira, H., and Harayama, S.: Bacteria belonging to the genus *cycloclasticus* play a primary role in the degradation of aromatic hydrocarbons released in a marine environment, Appl Environ Microbiol, 68, 5625-5633, 2002.

Kodama, Y., Stiknowati, L. I., Ueki, A., Ueki, K., and Watanabe, K.: *Thalassospira tepidiphila* sp. nov., a polycyclic aromatic hydrocarbon-degrading bacterium isolated from seawater, International journal of systematic and evolutionary microbiology, 58, 711-715, 10.1099/ijs.0.65476-0, 2008.

Konn, C., Charlou, J. L., Donval, J. P., Holm, N. G., Dehairs, F., and Bouillon, S.: Hydrocarbons and oxidized organic compounds in hydrothermal fluids from Rainbow and Lost City ultramafic-hosted vents, Chemical Geology, 258, 299-314, 2009.

Latimer, J. S., and Zheng, J.: The Sources, Transport, and Fate of PAHs in the Marine Environment, in: PAHs: An Ecotoxicological Perspective, John Wiley & Sons, Ltd, 7-33, 2003.
Lobbes, J. M., Fitznar, H. P., and Kattner, G.: Biogeochemical characteristics of dissolved and particulate organic matter in Russian rivers entering the Arctic Ocean, Geochimica et Cosmochimica Acta, 64, 2973-2983, 2000.
Ma, Y., Wang, L., and Shao, Z.: *Pseudomonas*, the dominant polycyclic aromatic hydrocarbon-degrading bacteria isolated from Antarctic soils and the role of large plasmids in horizontal gene transfer, Environ Microbiol, 8, 455-465, 10.1111/j.1462-2920.2005.00911.x, 2006.
Maruyama, A., Ishiwata, H., Kitamura, K., Sunamura, M., Fujita, T., Matsuo, M., and Higashihara, T.: Dynamics of microbial populations and strong selection for *Cycloclasticus pugetii* following the Nakhodka oil spill, Microb Ecol, 46, 442-453, 10.1007/s00248-002-3010-z, 2003.
McClintock, J. B.: Arctic: sea-ice loss sparks oil drilling boom, Nature, 479, 478, 10.1038/479478e, 2011.
McKew, B. A., Coulon, F., Osborn, A. M., Timmis, K. N., and McGenity, T. J.: Determining the identity and roles of oil-metabolizing marine bacteria from the Thames estuary, UK, Environ Microbiol, 9, 165-176, 10.1111/j.1462-2920.2006.01125.x, 2007a.
McKew, B. A., Coulon, F., Yakimov, M. M., Denaro, R., Genovese, M., Smith, C. J., Osborn, A. M., Timmis, K. N., and McGenity, T. J.: Efficacy of intervention strategies for bioremediation of crude oil in marine systems and effects on indigenous hydrocarbonoclastic bacteria, Environ Microbiol, 9, 1562-1571, 10.1111/j.1462-2920.2007.01277.x, 2007b.
Melcher, R. J., Apitz, S. E., and Hemmingsen, B. B.: Impact of irradiation and polycyclic aromatic hydrocarbon spiking on microbial populations in marine sediment for future aging and biodegradability studies, Appl Environ Microbiol, 68, 2858-2868, 2002.
Niepceron, M., Portet-Koltalo, F., Merlin, C., Motelay-Massei, A., Barray, S., and Bodilis, J.: Both *Cycloclasticus* spp. and *Pseudomonas* spp. as PAH-degrading bacteria in the Seine estuary (France), FEMS Microbiol Ecol, 71, 137-147, 10.1111/j.1574-6941.2009.00788.x, 2010.
Notomista, E., Pennacchio, F., Cafaro, V., Smaldone, G., Izzo, V., Troncone, L., Varcamonti, M., and Di Donato, A.: The marine isolate *Novosphingobium* sp. PP1Y shows specific adaptation to use the aromatic fraction of fuels as the sole carbon and energy source, Microb Ecol, 61, 582-594, 10.1007/s00248-010-9786-3, 2011.
Opsahl, S., Benner, R., and Amon, R. M. W.: Major flux of terrigenous dissolved organic matter through the Arctic Ocean, 8, American Society of Limnology and Oceanography, Waco, TX, ETATS-UNIS, 1999.
Ortega-Retuerta, E., Jeffrey, W. H., Babin, M., Bélanger, S., Benner, R., Marie, D., Matsuoka, A., Raimbault, P., and Joux, F.: Carbon fluxes in the Canadian Arctic: patterns and drivers of bacterial abundance, production and respiration on the Beaufort Sea margin, Biogeosciences, 9, 3679-3692, 10.5194/bg-9-3679-2012, 2012.
Proskurowski, G., Lilley, M. D., Seewald, J. S., Fruh-Green, G. L., Olson, E. J., Lupton, J. E., Sylva, S. P., and Kelley, D. S.: Abiogenic Hydrocarbon Production at Lost City Hydrothermal Field, Science, 319, 604-607, 2008.
Schmidt, C. W.: Offshore exploration to commence in the Arctic: can shell's oil-spill response plans keep up?, Environmental health perspectives, 120, A194-199, 10.1289/ehp.120-a194, 2012.
Schneiker, S., Martins dos Santos, V. A., Bartels, D., Bekel, T., Brecht, M., Buhrmester, J., Chernikova, T. N., Denaro, R., Ferrer, M., Gertler, C., Goesmann, A., Golyshina, O. V., Kaminski, F., Khachane, A. N., Lang, S., Linke, B., McHardy, A. C., Meyer, F., Nechitaylo, T., Puhler, A., Regenhardt, D., Rupp, O., Sabirova, J. S., Selbitschka, W., Yakimov, M. M., Timmis, K. N., Vorholter, F. J., Weidner, S., Kaiser, O., and Golyshin, P. N.: Genome sequence of the ubiquitous hydrocarbon-degrading marine bacterium *Alcanivorax borkumensis*, Nature biotechnology, 24, 997-1004, 10.1038/nbt1232, 2006.
Shao, Z., Cui, Z., Dong, C., Lai, Q., and Chen, L.: Analysis of a PAH-degrading bacterial population in subsurface sediments on the Mid-Atlantic Ridge, Deep Sea Research Part I: Oceanographic Research Papers, 57, 724-730, 2010.
Simoneit, B. R. T., Lein, A. Y., Peresypkin, V. I., and Osipov, G. A.: Composition and origin of hydrothermal petroleum and associated lipids in the sulfide deposits of the Rainbow field (Mid-Atlantic

Ridge at 36N), Geochimica et Cosmochimica Acta, 68, 2275-2294, 2004.
Sohn, J. H., Kwon, K. K., Kang, J. H., Jung, H. B., and Kim, S. J.: *Novosphingobium pentaromativorans* sp. nov., a high-molecular-mass polycyclic aromatic hydrocarbon-degrading bacterium isolated from estuarine sediment, International journal of systematic and evolutionary microbiology, 54, 1483-1487, 10.1099/ijs.0.02945-0, 2004.
Sorensen, S. R., Johnsen, A. R., Jensen, A., and Jacobsen, C. S.: Presence of psychrotolerant phenanthrene-mineralizing bacterial populations in contaminated soils from the Greenland High Arctic, FEMS microbiology letters, 305, 148-154, 10.1111/j.1574-6968.2010.01920.x, 2010.
Teramoto, M., Suzuki, M., Okazaki, F., Hatmanti, A., and Harayama, S.: *Oceanobacter*-related bacteria are important for the degradation of petroleum aliphatic hydrocarbons in the tropical marine environment, Microbiology, 155, 3362-3370, 10.1099/mic.0.030411-0, 2009.
Wang, B., Lai, Q., Cui, Z., Tan, T., and Shao, Z.: A pyrene-degrading consortium from deep-sea sediment of the West Pacific and its key member *Cycloclasticus* sp. P1, Environ Microbiol, 10, 1948-1963, 10.1111/j.1462-2920.2008.01611.x, 2008.
Whyte, L. G., Bourbonniere, L., and Greer, C. W.: Biodegradation of petroleum hydrocarbons by psychrotrophic *Pseudomonas* strains possessing both alkane (*alk*) and naphthalene (*nah*) catabolic pathways, Applied and environmental microbiology, 63, 3719-3723, 1997.
Witt, G.: Polycyclic aromatic hydrocarbons in water and sediment of the Baltic Sea, Marine pollution bulletin, 31, 237–248., 10.1016/0025-326X(95)00174-L, 1995.
Wu, J. Y., Jiang, X. T., Jiang, Y. X., Lu, S. Y., Zou, F., and Zhou, H. W.: Effects of polymerase, template dilution and cycle number on PCR based 16S rRNA diversity analysis using the deep sequencing method, BMC microbiology, 10, 255, 10.1186/1471-2180-10-255, 2010.
Yakimov, M. M., Giuliano, L., Denaro, R., Crisafi, E., Chernikova, T. N., Abraham, W. R., Luensdorf, H., Timmis, K. N., and Golyshin, P. N.: *Thalassolituus oleivorans* gen. nov., sp. nov., a novel marine bacterium that obligately utilizes hydrocarbons, International journal of systematic and evolutionary microbiology, 54, 141-148, 2004.
Yakimov, M. M., Timmis, K. N., and Golyshin, P. N.: Obligate oil-degrading marine bacteria, Curr Opin Biotechnol, 18, 257-266, 10.1016/j.copbio.2007.04.006, 2007.
Yuan, J., Lai, Q., Zheng, T., and Shao, Z.: *Novosphingobium indicum* sp. nov., a polycyclic aromatic hydrocarbon-degrading bacterium isolated from a deep-sea environment, International journal of systematic and evolutionary microbiology, 59, 2084-2088, 2009.
Yunker, M. B., and Macdonald, R. W.: Composition and Origins of Polycyclic Aromatic Hydrocarbons in the Mackenzie River and on the Beaufort Sea Shelf, Arctic, 48, 118 –129, 1995.
Yunker, M. B., Macdonald, R. W., Snowdon, L. R., and Fowler, B. R.: Alkane and PAH biomarkers as tracers of terrigenous organic carbon in Arctic Ocean sediments, Org Geochem, 42, 1109-1146, 2011.
Zaborska, A., Carroll, J., Pazdro, K., and Pempkowiak, J.: Spatio-temporal patterns of PAHs, PCBs and HCB in sediments of the western Barents Sea, Oceanologia, 53, 1005–1026., 2011.
Zheng, G. J., Man, B. K. W., Lam, J. C. W., Lam, M. H. W., and Lam, P. K. S.: Distribution and sources of polycyclic aromatic hydrocarbons in the sediment of a sub-tropical coastal wetland, Water Res, 36, 1457-1468, 2002.

Figure Legends

Fig. 1. Locations of the deep-sea sediment sampling sites in the high-latitude Arctic Ocean.

Fig. 2. The distribution and relative abundance of sixteen EPA-priority PAHs in the sediments of the Arctic Ocean. For each PAH, its relative abundance in all twelve samples was presented using different bar lengths in a sub-plot. The longest bar in a sub-plot indicates that this sample has the highest concentration value of a PAH in all twelve samples. The length of the other bars was proportionately shorted based on the ratios of the concentration values of other samples divided by the most abundant PAH in this sample. Acenaphthylene, acenaphthene and anthracene were not presented in this plot because their concentrations were below the detection limits in all sediment samples. U, M, and L in the sample names refer to the upper, middle, and lower layers of the sediments, respectively.

Fig. 3. Relative abundances of bacteria (genus level) in the sediments and enriched consortia from the Arctic Ocean. A: sediment; B: 4 ℃ consortia; C: 15 ℃ consortia; D: 25 ℃ consortia. The genera of abundance more than 1% of the total tags at least in one consortium were listed in each plot. “Others” refer to the genera constituting less than 1% of the total tags of a sample for all samples.

Fig. 4. PAH-removal extent of the consortia that were enriched from the sediments of the Arctic Ocean. The consortia were grown with a mixture of PAHs (naphthalene, phenanthrene, and pyrene) as the sole carbon and energy source. PAHs were quantified using GC/MS after pretreatments. Naphthalene was not included due to an error caused by its high volatility. Consortia names with prefixes “15” or “25” indicated they were enriched at 15°C or 25°C,

757 respectively.

758

759 **Fig. 5.** PCR-DGGE profiles of 12 PAH-degrading consortia that were enriched at 25 ℃. Lanes
760 1-3, site BN03; Lanes 4-6, site BN06; Lanes 7-9, site BN09; Lanes 10-12, BN12; and Lane 13,
761 negative control.

762

763 **Fig. 6.** PCR-DGGE profiles of PAH-degrading consortia that were enriched low temperatures.
764 Lane CK, negative control; A: consortia enriched at 4 ℃; and B: consortia enriched at 15 ℃.

765

766 **Fig. 7.** Growth curves of strains 15BN12U-14 and 15BN12L-10 using individual PAHs as the
767 sole carbon and energy source in ONR7a medium. A and B: strain U-14 and L-10 cultivated at
768 15 ℃, respectively. OD_{600} was defined as meaning optical density at 600 nm.

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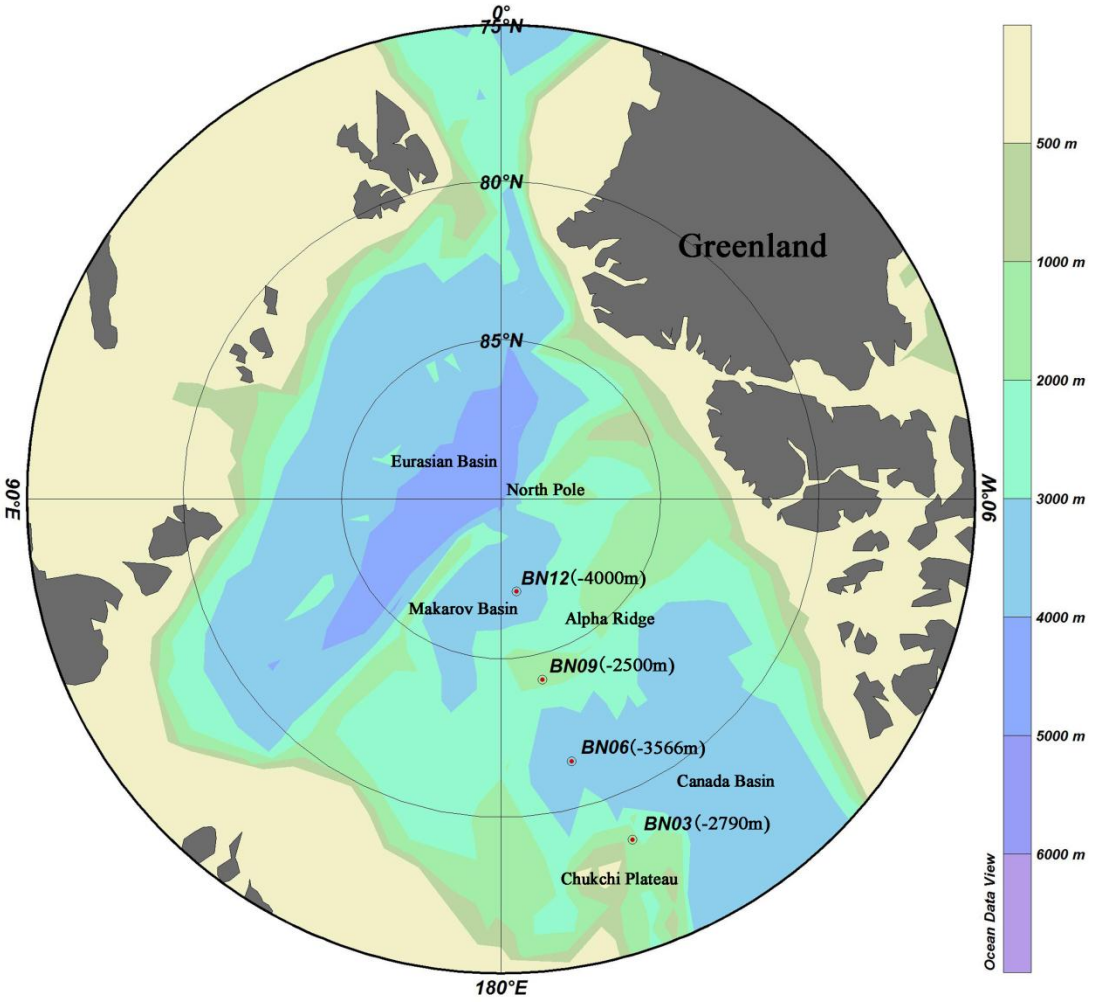
770

771 Fig.1



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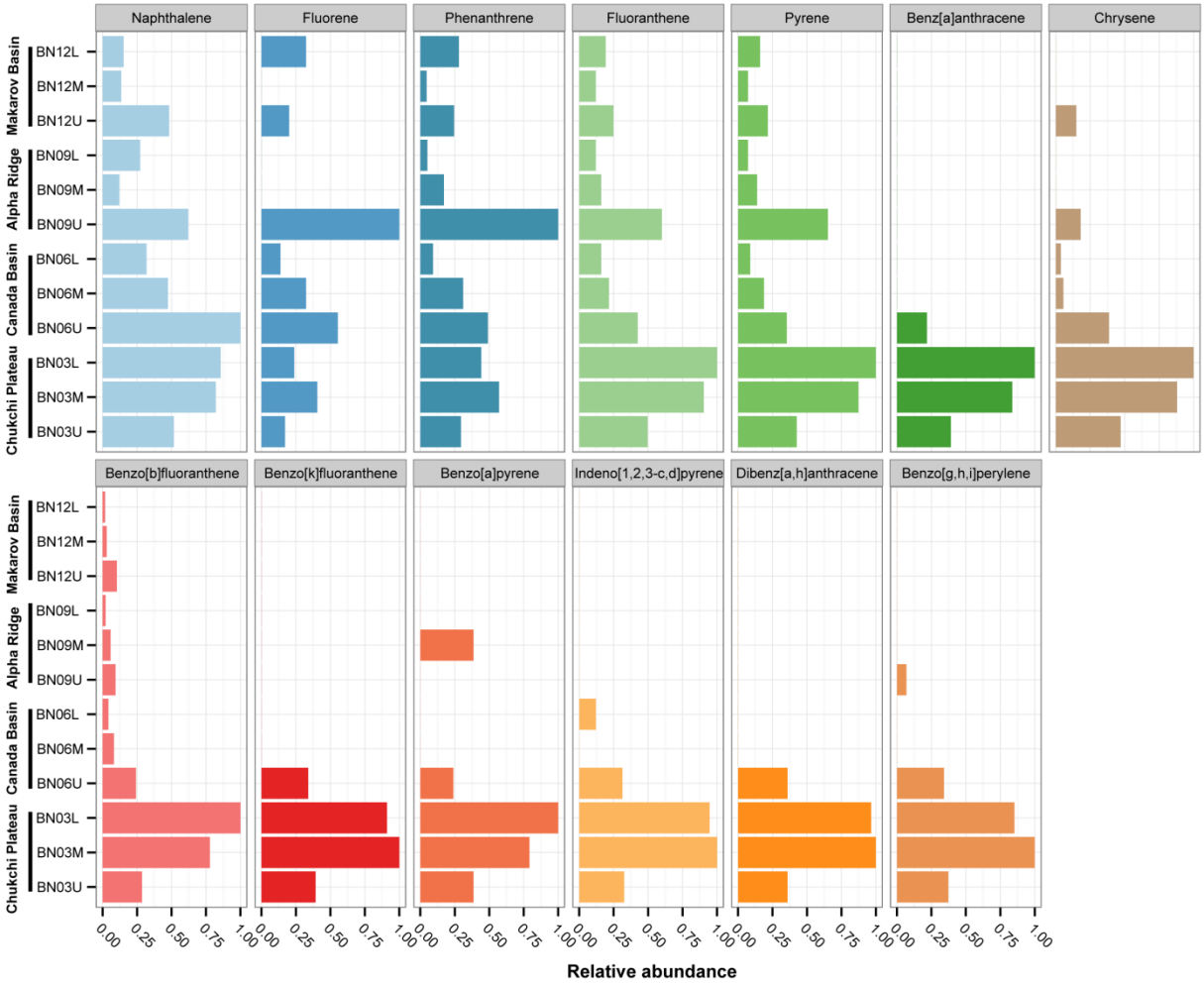
774 Fig. 2



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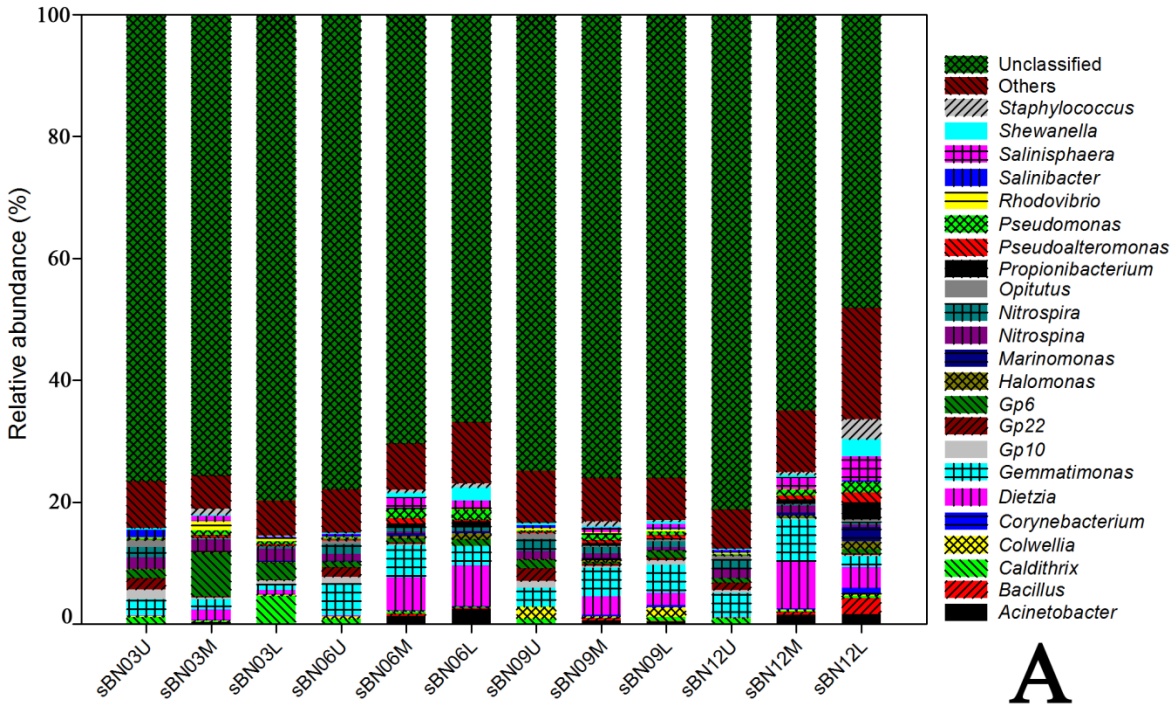
777 Fig.3



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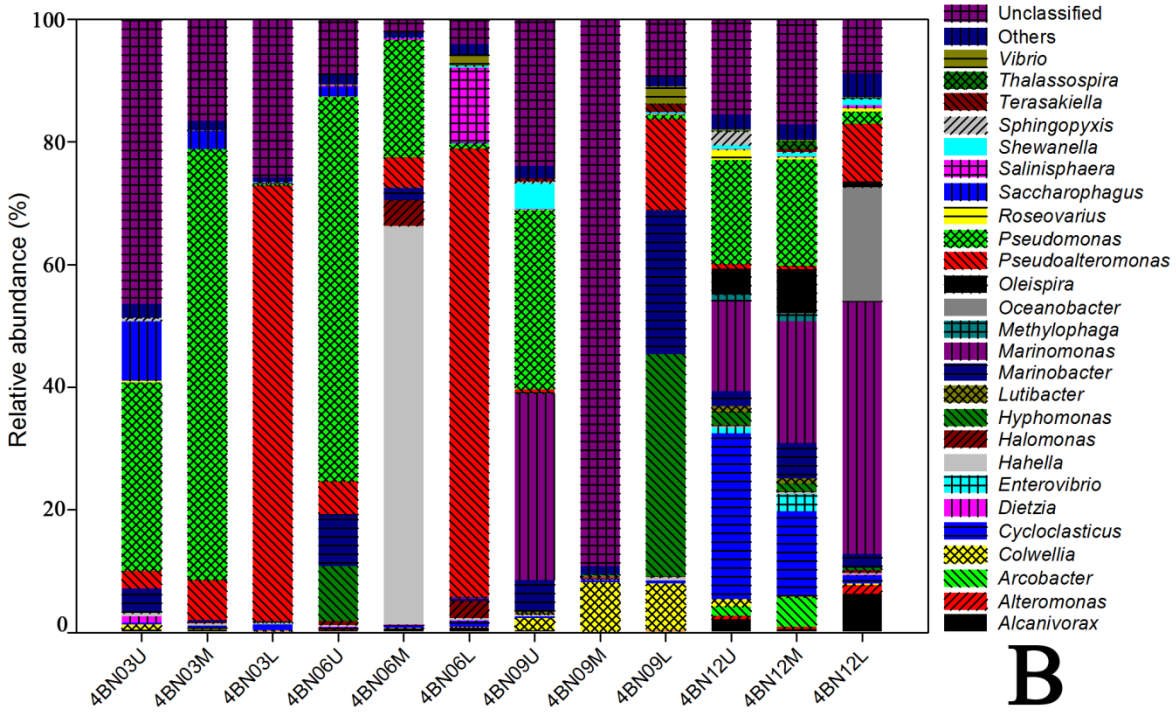

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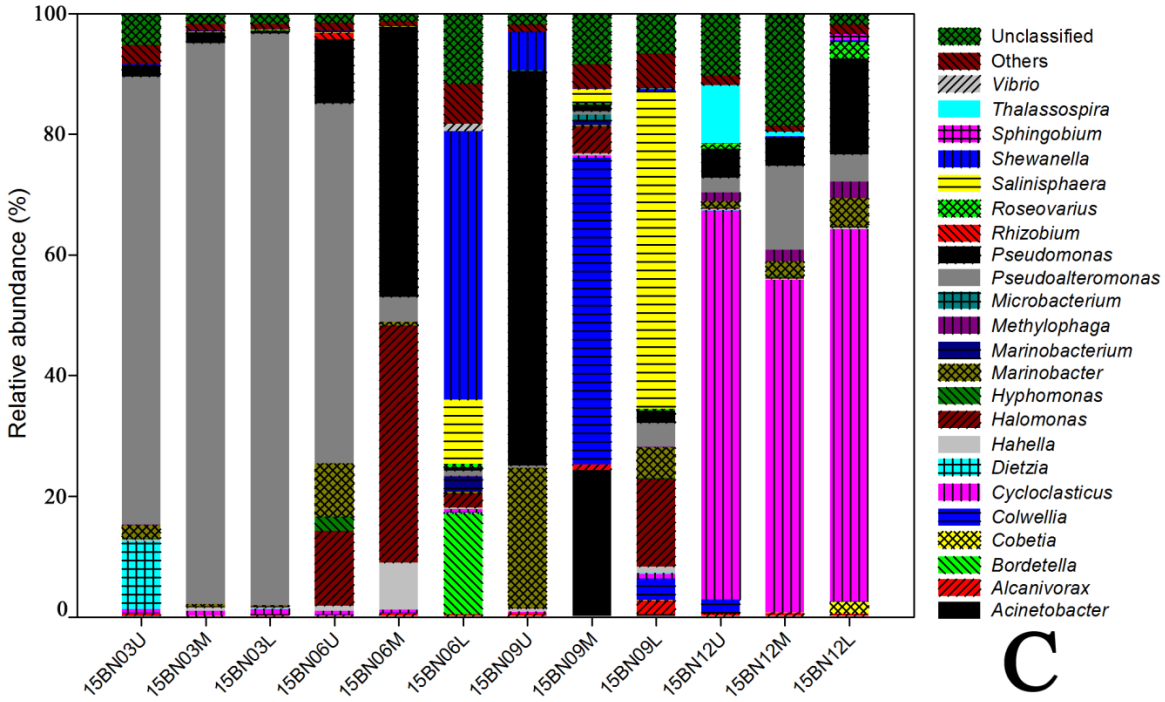
780 Fig.3 (continue)



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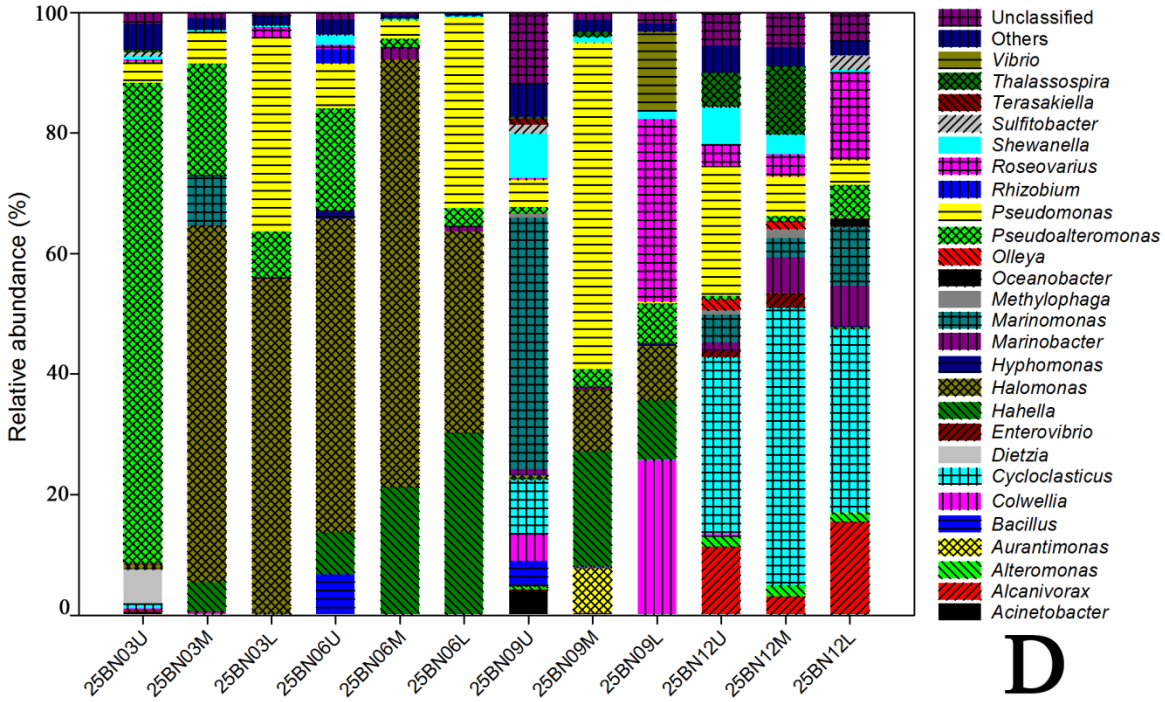

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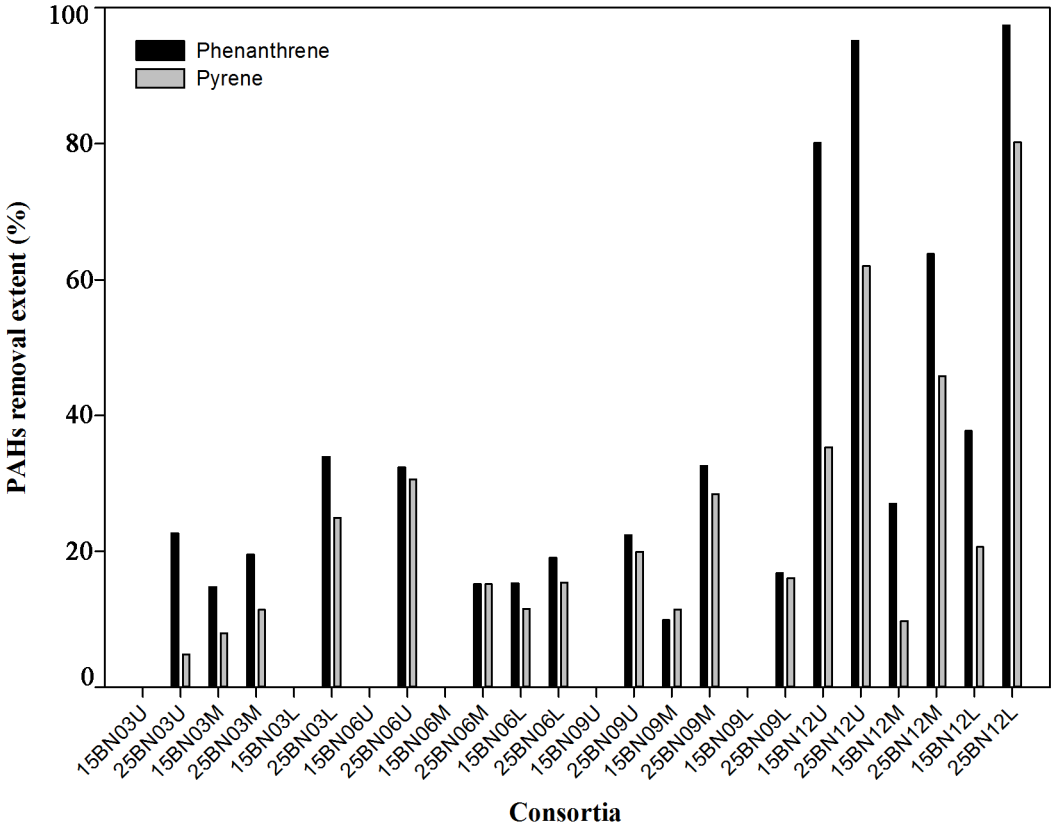
783 Fig. 4



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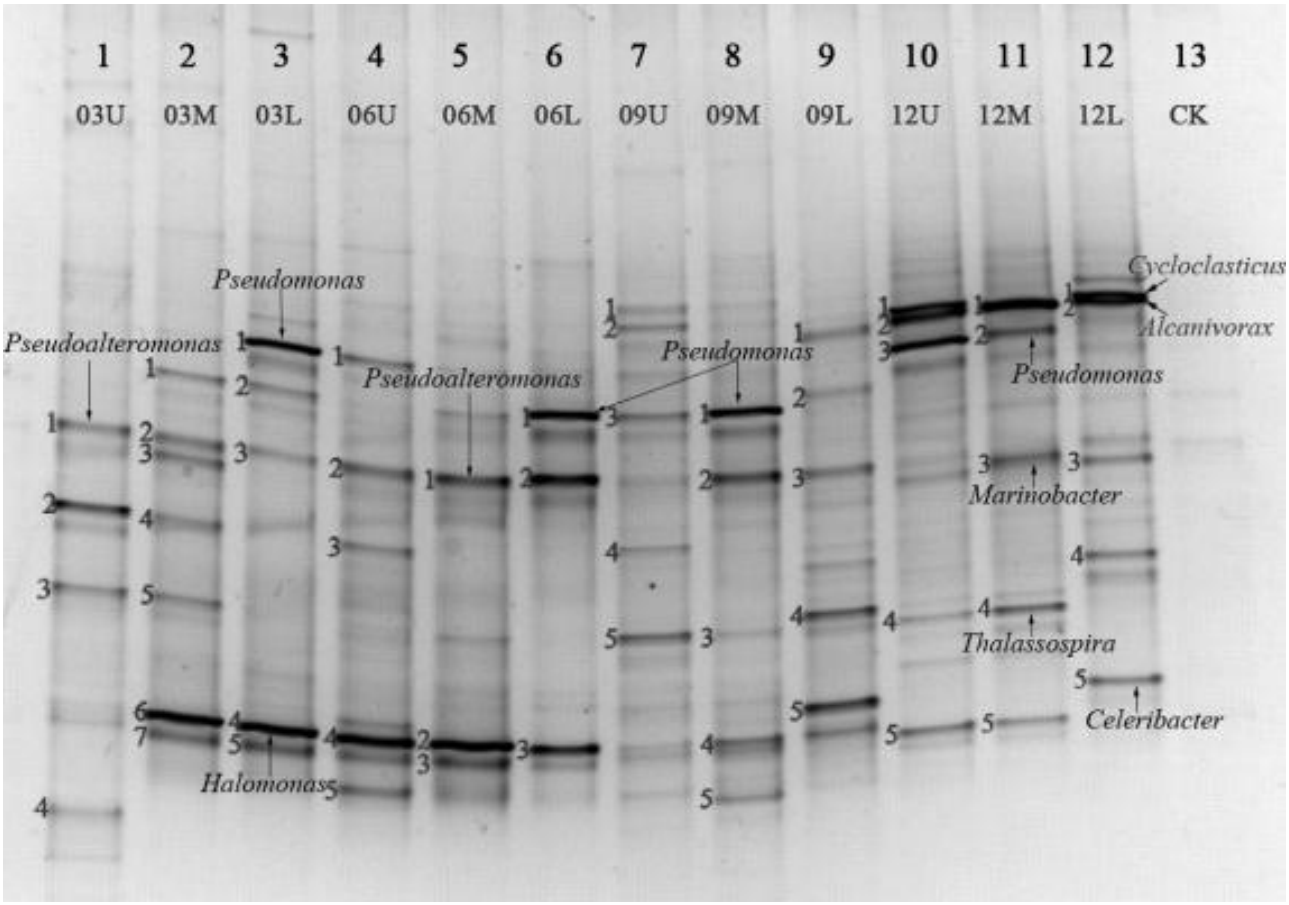
786 Fig. 5



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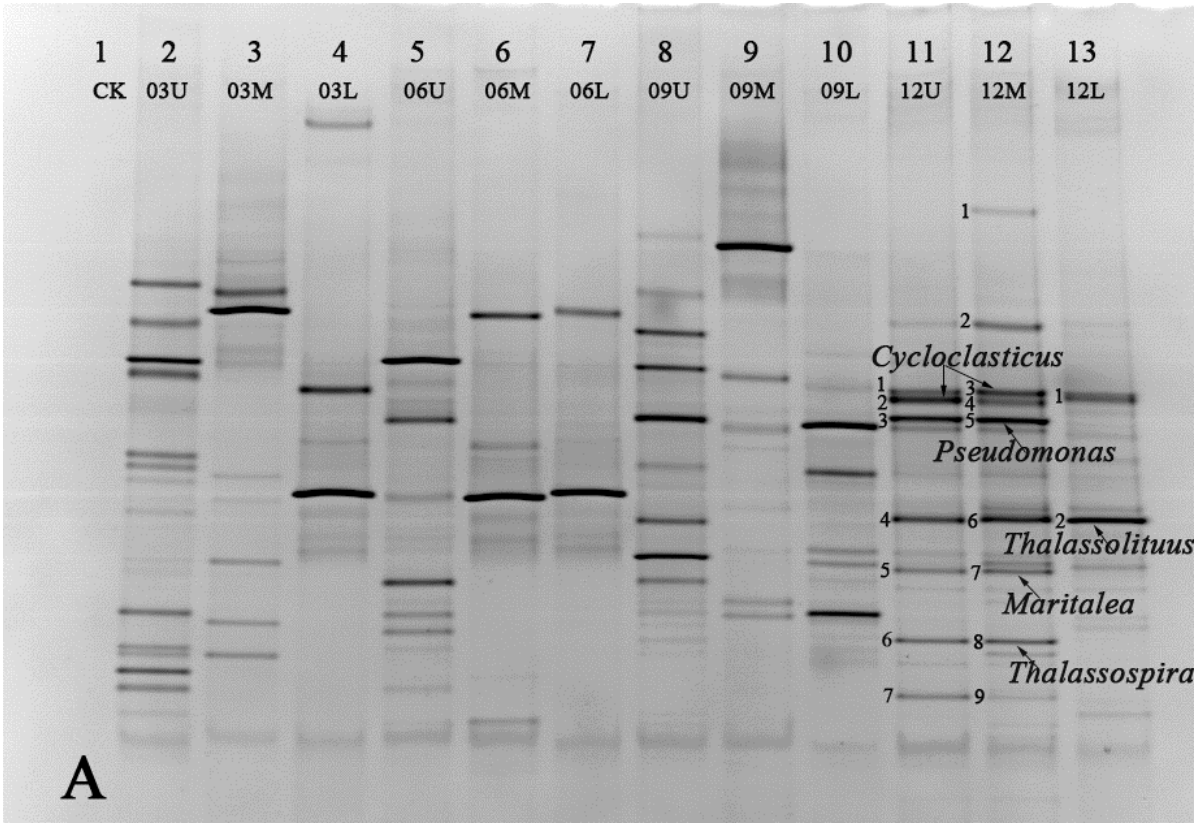
789 Fig. 6



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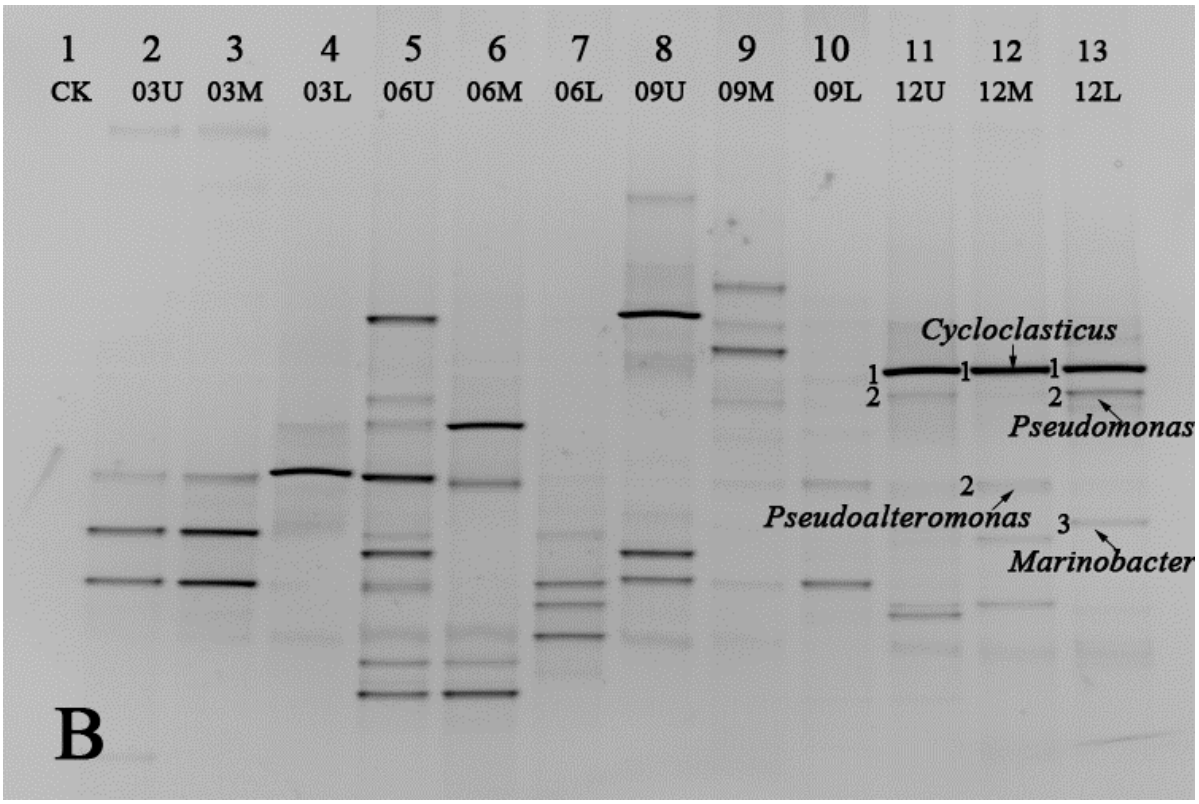

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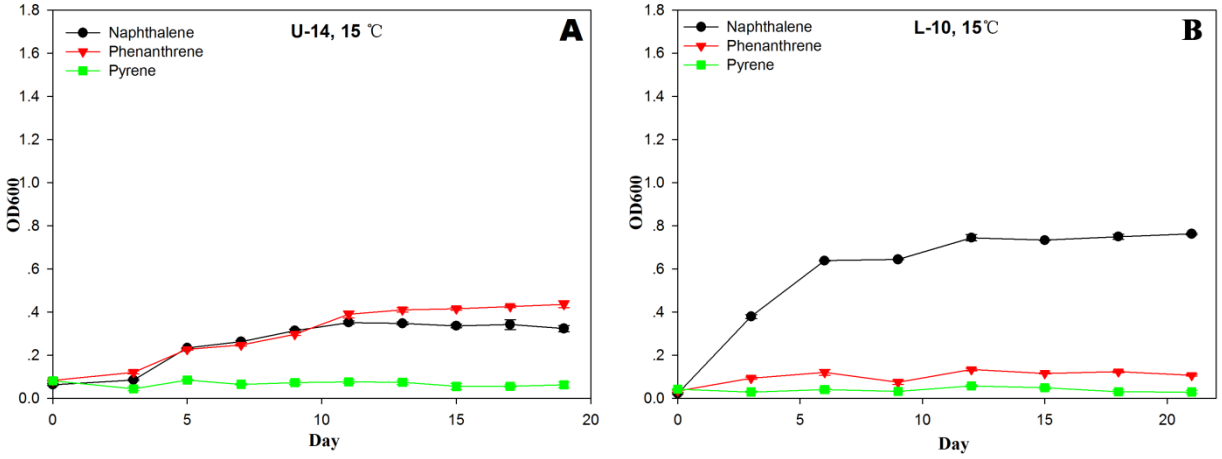
792 Fig. 7



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