Dear Editor，
Thank you for handling our manuscript.
We also want to thank the three referees for their thoughtful comments and suggestions to improve the manuscript much.

Please find below our response to the referee's comments. All the comments and suggestions were taken into consideration in the revised version of the manuscript. All the changes made are highlighted in blue in the revised manuscript and Supplemental Material.

In addition to the response to referees, the manuscript was submitted to *Nature Publishing Group Language Editing* company (Submission ID: DG2D9CX8) for language editing before revised submission.

Best regards,
Zongze Shao

Replay to referee Dr. Andrew Steen,

Thanks for your positive comments on our manuscript. Your questions were responded as follows:

R.: With respect to the writing, the manuscript is well-organized and the structure is logical, but there are quite a few superficial grammatical errors that should be fixed.
A.: The language and grammar of this manuscript will be edited by *Nature Publishing Group Language Editing* Company before it is resubmitted.

R.: P 13986 line 2: change to "tend to accumulate"
A.: We will change these words in the revised manuscript. Thanks.

R.: P 13986 line 13 change to "The potential degraders including..., with Dietzia as the most abundant, occurred in all sediment samples."
A.: Agree. Thanks.

R.: P 13987 line 1 change to "and is almost"
A.: OK. Thanks.

R.: P 13987 line 3 change to "Loading of terriginous organic carbon through rivers into the Arctic Ocean, and the influence of that terriginous organic matter on bacterial diversity in coastal water, have drawn attention". (Keep the same citations).
A.: Will be changed accordingly, thanks.

R.: P 13987 line 16: change to "have increased"
A.: Agree. Thanks.

R.: P 13988 line 5: Change to "Biotic and abiotic processes, as well as top-down processes (suspended particle absorption and sinking) and bottom-up processes (thermogenesis below the surface) are all believed to contribute to the accumulation of PAHs (reference)." * Also: I am not aware of biotic processes that are known to produce PAHs. * Also: to me, "top-down" and "bottom-up" processes are ecological terms referring to predator or prey/nutrient control over populations. The authors are using them here to refer to the physical top or bottom of the water column. I encourage them to find different terms.
A.: Thanks for your questioning. Here, we want to explain the PAHs sources in deep-sea sediment. According to previous reports, the main sources of PAHs are long-range atmospheric transport and abiogenic production in some special deep-sea environments, such as hydrothermal vent.

So, we modified this sentence as "long-range atmospheric transport and abiogenic production in deep-sea hydrothermal vents are believed to contribute to the accumulation of PAHs (Friedman, C. L. *et al.*, 2012; Proskurowski, G., *et al.* 2008; Simoneit, B. R. T., et al. 2004; Konn, C., *et al.* 2009)."

R.: P 13988 Line 28: Change to "To the best of our knowledge,"
A.: Revised, thanks.

R.: p 13991 * Line 12: Is "consortia" the correct term here? I understand "consortium" to mean a well-defined association of a small number of species, for instance the association between archaea and sulfate reducers that drives anaerobic methane oxidation. The authors have more complex mixtures of microorganisms in which many of the species are probably involved in PAH degradation, but some may not be and it is possible that none are essential to PAH degradation. For that reason, I prefer a term like "enriched communities"
A.: We agree with your opinion and will change “consortia” to “enriched cultures” in the revised manuscript.

R.: p 13992 Line 22: The authors should post this script as a supplemental file as a service to other authors working on similar projects.
A.: This script (combine.bipes.pl) is written by our co-author Ms. Huafang Sheng using Perl language, as below. Also, this script is added in the Supplemental Materials.

During our IHTS data analysis process, all clean reads of each sample were stored in an independent “fasta” format file. Before executing this script, user should build a file named “fa.list” for storing the file paths of all these “fasta” format files. Then, the user can use the command “perl combine.bipes.pl fa.list outprefix” to execute the script. Finally, clean reads of each sample were combined into a file named “outprefix.combined.fa”.

```
#!/usr/bin/perl -w
use strict;

if ($#ARGV ne 1)
{
 print "perl $0 <fa.list> <outprefix>\n";
 die;
}

open I,"$ARGV[0]" || die "can not find I:$!";
my $out = "$ARGV[1].combined.fa";
open OUT,">$out" || die "can not open OUT:$!";


while(<I>)
{
 chomp;
 open II,"$_" || die "can not find II:$!";
```

```
#my @m = split/\./;
#pop @m;
my $sample = $_;
my $n = 0;
while(<II>)
{
  my $seq = <II>;
  chomp $seq;
  $n++;
  print OUT ">$sample\_$n\n$seq\n";
  }
}
```

R.: p 14000 * Line 22: I do not know what the theory of global distillation effects is. It must be cited, and the authors should briefly explain what it posits.

A.: First of all, you concern located in line 15. Wiki gives an explanation of the theory as follows,

Global distillation or the grasshopper effect is the geochemical process by which certain chemicals, most notably persistent organic pollutants (POPs), are transported from warmer to colder regions of the Earth, particularly the Poles and mountain tops. Global distillation explains why relatively high concentrations of POPs have been found in the Arctic environment and in the bodies of animals and people who live there, even though most of the chemicals have not been used in the region in appreciable amounts. (http://en.wikipedia.org/wiki/Global_distillation).

This theory was firstly proposed by Dr. Goldberg in 1975. Actually, we have cited relative references in line 17.

For more accuracy, we will change this sentence to “According to the theory of “global distillation””, in the revised manuscript.

R.: p 14001 Line 28: Omit "by"

A.: We will delete this word in the revised manuscript.

R.: p 14002 Line 2: "must" is too strong. Change this to something like "It seems likely that they play an important role"

A.: Thanks. We will change this sentence as your advice in the revised manuscript.

R.: p 14002 Line 11: Omit "The" before "Cycloclasticus"

A.: Deleted, thanks.

R.: p 14002 Line 29: Change "more kinds of carbon source" to "more distinct carbon compounds"

A.: These words will be changed in the revised manuscript.

R.: p 14004 Line 7: Change to "It is worth mentioning that"
A.: Have changed in the revised manuscript. Thanks.

R.: Figure 2: Stacked bar graphs such as this one are common, but they are not the best way to display data such as these, because it is very difficult to assess changes in the height of the bars that are in the middle of the plot. A better way would be to make a separate panel for each genus, as, for instance, shown in the "panel bar chart". Examples of such a chart (with code using the open-source statistical platform R) is given here: http://timotheepoisot.fr/2013/02/17/stacked-barcharts/.
A.: First of all, we really appreciate you offering us a better way to display our data. We have redrawn this figure as below.
Figure 2 legend:
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.

R.: * Figure 4: "%" does not make sense as a unit for something labeled "removal rate": a rate needs to have units of something per unit time. Does it mean "% loss over the entire incubation"? Neither the main text nor the figure legend adequately explain this calculation. In any case, better units would be g PAH per liter per hour. Also, change "apt" to "high" in the figure legend.
A.: Yes, it does. "%" just means that a certain of PAH consume percentage over the entire incubation.

As your advice, we added a description of the calculation of PAHs removal rate in "*2.5 PAH-removal rate quantification by GC-MS*" as below:
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 rate was calculated according to the formula:

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

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.

Additionally, the word "apt" also changed into "high" in the revised manuscript.

Replay to referee Dr. Kelly McFarlin,

Thank you for reviewing our manuscript, and all the constructive comments and suggestions. Your comments and questions were responded point by point.

R.: I'm happy to hear that the authors plan to improve the manuscript by submitting it for "writing improvement". There are many cases throughout the manuscript where the language needs improvement. There are too many cases to cite in this review.
A.: We will submit it to *Nature Publishing Group Language Editing* Company for language editing before submitting the revised manuscript.

R.: Authors discuss terrigenous and anthropogenic PAHs sources throughout the Introduction and Discussion. It is unclear how terrigenous sources relate to the present study. I encourage the authors to clarify their argument.
A.: Terrigenous sources certainly contribute to the distribution of PAHs in the Arctic Ocean. Previous studies showed that terrigenous PAHs could be originated from decaying peat products and plant detritus, and transported to the Arctic Ocean by rivers (Dahle, *et al*. 2003. *Sci Total Environ* 306(1-3): 57-71; Yunker, *et al.* 1993. *Geochimica et Cosmochimica Acta* 57(13): 3041-3061). Moreover, Yunker *et. al.* (2011) indicated that natural origin PAHs (such as petrogenic PAHs from peat and plant detritus) dominated in Arctic Ocean sediments compared to anthropogenic combustion PAHs, especially in Arctic coastal seas (Yunker *et al.* 2011. *Org Geochem* 42(9): 1109-1146). Even in the remote areas, we cannot exclude the terrigenous PAHs transfer via ocean currents or sea ice transport. Thus, we believe that the PAHs determined in this study contained both terrigenous and anthropogenic origins, which all could be served as carbon sources for various PAH-degrading bacteria.

In order to clarify our purpose, we inserted a sentence at the end of the fifth paragraph in the "*Introduction*" section of the revised manuscript as follow "Therefore, these allochthonous PAHs could serve as carbon sources for various PAH-degrading bacteria in the Arctic sediments".

R.: I disagree with author's generalized statement that polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants (POPs). Many PAHs are easily biodegradable. High molecular PAHs can be resistant to bioremediation, but I am highly skeptical that all PAHs found in petroleum are classified as POPs. I have not found any literature to support this claim and the authors have not provided any references to this statement.
A.: Thanks. We agree with this suggestion. We checked the Stockholm Convention (http://chm.pops.int/TheConvention/ThePOPs/ListingofPOPs/tabid/2509/Default.aspx) and found that PAHs are not included in the POPs list.

Thus, we changed the description accordingly, such as the first sentence in the ***Abstract*** as "Polycyclic aromatic hydrocarbons (PAHs) are common organic pollutants", and the sentence "which belong to persistent organic pollutants and tend

to accumulate in marine sediments (page 13987 line 8)" as "PAHs tend to accumulate in marine sediments …" in the revised manuscript.

R.: Why were samples collected from the Chukchi Plateau Makarov Basin? Please include a statement of the significance of this location.
A.: This work mainly aimed to examine the distribution of PAHs and PAH-degrading bacteria in deep-sea sediments of the high-latitude Arctic Ocean. We suppose that different regions possess different patterns in PAHs and the degrading bacteria, which are suffered from varied influences of both hydrography and terrigenous inputs. Thus, these four typical geographical regions were chosen in this survey.

For further clarification, we rephrased the sentence (lines 13-15 in page 13989) as "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)".

R.: Were oxygen concentrations measured in the sediments during sampling? In the incubations? It is unclear what in situ conditions were represented in the incubations.
A.: The *in situ* oxygen concentrations were not measured, as no such equipment was available during the sampling. Incubations were carried out aerobically not only on board but also in our laboratory (see "*2.4 PAH-degrading bacteria enrichment*" in page 13991).

In this report, we did not intend to replicate all the *in situ* conditions but to reflect the occurrence of PAH-degrading bacteria therein. Moreover, as bacterial growth with PAHs is quite slow, we think it is not necessary to measure oxygen concentration during the incubation, especially the incubations were continuously agitated in the rotary shakers.

R.: Pg 13987, line 25. Paragraph is about PAH contaminants, but it appears that references concerning terrigenous organic carbon are used (e.g. Yunker et al., 2011). Please clarify the source of the PAHs and be sure to not use references concerning terrigenous PAH distribution for proof of anthropogenic contamination.

Be sure to identify abbreviations at the first occurrence (e.g. dw).
A.: This paragraph is to describe distributions of PAHs in the Arctic Ocean sediment, rather than PAH contaminants. The words "PAH contaminants" might lead to misunderstanding, so we deleted the word "contaminants" in line 22 of page 13987, instead of changing the references. PAHs origin in the Arctic regions was introduced in the following paragraph.

"dw" have been spelt out in the revised manuscript. Thanks.

R.: Pg 13989, Sediment Collection. What was the water depth at these sampling locations? What was the temperature? Were in situ nutrient concentrations measured?
A.: The water depth of sites BN03, BN06, BN09 and BN12 are 2790m, 3566m, 2500m and 4000m, respectively. These data are provided in Supplement Table S1.

Unfortunately, the *in situ* temperature of the sediments was not determined. Generally, the temperatures of deep seafloor range from -1^{o}C to 4^{o}C (Jorgensen, B. B. and A. Boetius, 2007. *Nat. Rev. Microbiol.* 5(10): 770-781). The nutrients concentrations in these sediments weren't measured. But, in our another report (under review), we determined the concentrations of other nutrient factors, such as total carbon (TC), total nitrogen (TN), total phosphorus (TP) and total organic carbon (TOC). These data showed that TN (*P*=0.013), TP (*P*=0.014) and TOC (*P*=0.017) in the sediments (including the sediments used in this study) of the Canadian Basin were significantly lower than those from Chukchi Shelf, indicating that the Basin is more oligotrophic compared to the Shelf.

R.: Pg 13990, line 10. Please describe the ONR7a enrichment medium.
A.: Have added a description in the revised manuscript.

R.: Pg 13990, line 17. Were spiked surrogate standards used to calculate extraction efficiency? Additionally, please describe how PAHs were quantified with the internal standard method and identify the internal standard.
A.: Yes. The recoveries for surrogate standards were 81.6-105.2%.

The internal standard used in this study is m-terphenyl. We have supplemented this information in line 6 of page 13990 as "……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".

After confirming with the co-author Dr. Liping Jiao, target PAHs quantification details were described below (supplemented information are highlighted with underline).

"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. In brief, 0.05 mL of the 1 mg L^{-1} surrogate standard mixture solution was spiked into 20 g of freeze-dried sediment before 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."

We will supplement this information descripted above in "*2.3 PAHs quantification of deep-sea sediments*" of the revised manuscript.

R.: Pg 13991, line 1. Five grams of soil were spiked with a PAH mixture in addition to 1 mL of crude oil. The high concentration of oil added to the soil enrichment in addition to the PAH mixture may have caused the slow growth of PAH-degrading bacteria. Please comment on this.

Were oxygen concentrations monitored in the enrichments? Were the enrichments left open to the atmosphere? If oxygen was monitored, how do these levels correspond to natural concentrations? If oxygen was not monitored, discuss the limitations of the enrichments cultures.

A.: We agreed that at the initial round enrichment, alkane degraders would outgrow the PAH ones, but PAHs degraders still will be enriched, as crude oil also contains various PAHs more diverse than we added. In addition, we supplied more PAHs in the crude oil that is served as the solvent to disperse PAHs and to enhance their homogeneity and bioavailability in the enrichments. According to our Illumina high throughput sequencing (IHTS) results (Fig. 3, Supplemental Material Table S5), PAH-degrading bacteria of *Pseudomonas*, *Pseudoalteromonas*, *Cycloclasticus*, and *Halomonas* always were dominated at 4^{o}C, 15^{o}C and 25^{o}C enrichments no matter what mixture carbon source or sole PAHs was used.

Oxygen concentrations weren't monitored in the enrichments. In our opinion, oxygen concentrations will not fell below the limitation to support the bacterial growth for the purpose of initial enrichment. As we presented in the manuscript, especially under agitation conditions in lab, we believed that oxygen is not limitation for PAH bacterial growth. Moreover, the dominant PAH-degrading bacteria mentioned above are regarded as aerobic PAH degrading bacteria in literatures.

R.: Pg 13991, line 10. Explain 'repeated twice'. It is unclear what part was repeated twice. How do the nutrient concentrations in the mineral medium correspond to the natural conditions found in the sediments?

A.: 'repeated twice' means that the 4^{o}C enriched cultures were transferred into fresh media and enriched twice at 15 ℃ and 25 ℃, respectively, in order to obtain the stable PAH-degrading bacterial community for subsequent analysis.

We didn't measure *in situ* nutrients concentrations of these sediments. As we presented above, this study did not intend to replicate all the *in situ* conditions but to reflect the occurrence of PAH-degrading bacteria therein. We agree that nutrients in the enrichments are higher than those *in situ*, and this would lead to the change of bacterial community structures compared *in situ*. To detect the *in situ* bacterial community structure, we adopted 16S RNA gene sequencing of the sediments, which

supplies a background of the *in situ* bacterial diversity. All these results combined together would illustrate the distribution pattern of PAH degrading bacteria.

R.: Pg 13991, line 20. It is unclear how PAHs were quantified. Please include a detailed description of quantification. Were abiotic losses of PAHs calculated in the incubations? It is unclear how the % losses of PAHs were calculated and if abiotic losses were accounted for.

A.: After concentration and purification of the extracts from the enrichment cultures and uninoculated controls according to the procedures descripted in "*PAHs quantification of deep-sea sediments*", the residual PAHs were quantified using the external standard method.

Firstly, a series of dilutions (0.2-2 ppm) of PAHs standard mixture was carefully prepared and standard curves for naphthalene, phenanthrene and pyrene were made, respectively.

Then the residual PAHs in the enrichment cultures and uninoculated controls were analyzed. The recovery rates of naphthalene (28.7%), phenanthrene (53.7%) and pyrene (57.5%) were then calculated by the quantity difference before and after enrichment, extraction and purification in the uninoculated controls. Then they were used for assessing abiotic losses and the losses during the extraction and purification processes. In this study, naphthalene-removal rate was not calculated due to the low recovery which may be attributed to its high volatility.

Finally, PAH-removal rate was calculated as follows:

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

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.

We will insert these quantification information descripted above in "*2.5 PAH-removal rate quantification by GC-MS*" of the revised manuscript.

R.: Pg 13993, line 10. Why were the bacteria isolated at 15°C chosen as the representative culture?

Could the concentration of PAHs measured in the sediments be the results of natural oil seeps?

A.: According to IHTS and PCR-DGGE results (Fig. 3C and Fig. 6B), *Cycloclasticus* is dominated in the 15 ℃ enriched cultures, particularly in the cultures from BN12 site. Thus, we chose 15 ℃ enrichment cultures as the representatives to isolate *Cycloclasticus.*

We could not exclude that PAHs were originated from natural oil seeps, especially in the coastal seas of the Arctic, because the assessment made by the US Geological Survey has shown that approximately 30 and 13% of the world's undiscovered gas and oil reserves, respectively, may be found in the Arctic region.

R.: Pg 14000, line 3. Please provide the concentration of phenanthrene.

A.: Added.

R.: Pg 14002, line 1. It is incorrect to say that the genus occupied 0.2-0.5% of the total bacteria in each sample. It is correct to say that the genus occupied 0.2-0.5% of the total bacteria sequenced…
A.: Corrected. Thanks.

R.: Pg 14002, line 11. 'The *Cycloclasticus* bacteria were found in all these samples…' Please define 'these samples'. It is unclear what samples you are talking about.
A.: 'these samples' refers to the twelve *in situ* sediment samples. We have changed this sentence as "*Cycloclasticus* bacteria were found in all twelve of the *in situ* sediment samples, ……".

R.: Pg 14002, line 23. '…were also found as dominant members in some PAH-degrading consortia in this report'. Please describe where they were found.
A.: Because the Supplemental Material Table S5 has presented the most abundant bacteria in the 4^{o}C, 15^{o}C and 25^{o}C consortia, we appended "(see Supplemental Material Table S5)" behind "……were also found as dominant members in some PAH-degrading consortia in this report" in the revised manuscript.

R.: Pg 14003, line 4. Please describe 'the first two bacteria'.
A.: The first two bacteria referred to *Marinomonas* sp. D104 and *Sphingobium* sp. C100. So we rephrased the sentence "Genome sequencing revealed the degradation genes for PAH degradation in the first two bacteria (Dong et al., 2014b, a)." as "Genome sequencing revealed that strains D104 and C100 possessed several genes involved in the initial hydroxylation and intermediate metabolic steps of PAHs (Dong et al., 2014b, a)".

R.: Pg 14003, line 12. Please insert 'sequenced' after 'total bacteria' or something similar. Please discuss the relevance of incubating Arctic sediments at 15^{o}C and 25^{o}C.
A.: Inserted. Thanks.

The reason of choosing 15 ℃ is to select the psychrotolerant PAH-degrading bacteria which grow very slowly under 4 ℃, but usually have optimal growth temperatures approximate 15 ℃. Thus, we shifted their enrichments to 15 ℃ instead of 4 ℃. The enrichment under 25 ℃ is a parallel treatment to observe the temperature effect on the community structure. Despite the variation occurred to some extent, but the dominant PAH-degrading bacteria like *Pseudomonas*, *Cycloclasticus*, *Halomonas* and *Pseudoalteromonas* still remained to be the predominant at 25 ℃ (Fig 5 and Supplemental Material Table S5).

Replay to Anonymous Referee #3,

We appreciate your constructive suggestions. Your comments and questions were responded as follows.

R.: The authors should highlight the importance of the degradation of PAHs in situ at low temperatures and high pressures. From the physical point of view PAHs degradation is more difficult under these environmental conditions.

It is not understandable why the authors have included in the experimental design, bacterial growth at 15 and 25C. There are many previous works that has study the PAHs degradation at high temperatures.

A.: We agree with your suggestion that deep sea environmental conditions like low temperature and high pressure should be taken into consideration to understand the role of PAH-degradation in the sediments. Another group of our lab starts to mimic the deep sea conditions now and has confirmed PAH degradation under high pressure though the bacteria were initially isolated under normal atmospheric pressure (unpublished). We also start to enrich PAH-degrading bacteria *in situ* in deep sea water columns at approximately 3000m depth in both Indian Ocean and South China Sea, but enrichments are ongoing without initial results.

As to the low temperature, we have carefully considered about its effect. Initially, PAH-degrading communities were enriched under 4^{o}C, and then were shifted to 15^{o}C to speed up the growth of psychrotolerant PAH-degrading bacteria. Finally, they were incubated under 25^{o}C to observe the effect of temperature increase on community structure. Generally, psychrotolerant bacteria grow better with temperature increase, and have optimal growth temperature approximate 15^{o}C. Hence, we think that enrichments of psychrotolerant PAH-degrading bacteria can happen under both 4^{o}C and 15^{o}C.

R.: There are very interesting data as diversity index (Shannon index) which have been barely discussed.

A.: Thank you for your suggestion. We discussed further at the end of the third paragraph of "Discussion" section as follows.

"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".

R.: The current manuscript includes deep sediment samples (4000-2500 m) from approximately 250-400 atmospheres of pressure. However, it has not mentioned the effect of the high pressure changes that samples are submitted.

A.: As mentioned above, we believe that the PAHs degraders could conduct PAH degradation under high pressures of *in situ* water depth. Next, we will compare the efficiency of different strains of the same species which are originally isolated from

surface water (or coastal areas) and deep sea, respectively, in PAHs degradation under high pressures.

R.: It is not explained in the discussion why the The top-down concept is not well used. This concept is used to refer the depredation control of the processes.
A.: Thank you for your question. The similar question was also raised by the referee Dr. Andrew Steen. We agree that "top-down" is an ecological term referring to predator or prey/nutrient control over populations. Here, we use it (page 13988, line 6) to explain the PAHs sources in deep-sea sediment.

According to previous reports, the main sources of PAHs are long-range atmospheric transport and abiogenic production in some special deep-sea environments, such as hydrothermal vent. So, we modified the sentence (page 13988, lines 5-8) as "Long-range atmospheric transport and abiogenic production in deep-sea hydrothermal vents are believed to contribute to the accumulation of PAHs (Friedman, C. L. *et al.*, 2012; Proskurowski, G., *et al.* 2008; Simoneit, B. R. T., et al. 2004; Konn, C., *et al.* 2009)." in the revised manuscript.

Correspondingly, the origin and distribution of PAHs in the Arctic deep sea sediments was discussed in the second paragraph of the "Discussion" section.

R.: 2.8. Bacteria isolation, identification and phylogenetic analyses. Why 35 cycles for the PCR? The authors should test if that number is too low or too high to avoid amplification of not desirable DNA.
A.: Thirty-five cycles were only performed in the Rep-PCR. We agree that the cycles are more than normal PCR. However, according to our experiences, genome fingerprinting is not easy to be obtained by amplifying under 30 cycles or less. We usually perform 35 cycles in the Rep-PCR analysis.

R.: 3.3 PAH degradation of PAH enrichment consortia. The first paragraph of this section should be in material and methods.
A.: we agree and delete the first sentence of this paragraph in the revised manuscript. Thanks.

R.: 3.4 community structures of the consortia enriched with PAHs. I think there is a mistake in the figures.
A.: Sorry, we cannot find the mistake in Fig.3b-3d. Would you please specify it?

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

Chunming Dong[1¶], Xiuhua Bai[1, 4¶], Huafang Sheng[2], Liping Jiao[3], Hongwei Zhou[2] and Zongze Shao[1 #]

[1] Key Laboratory of Marine Biogenetic Resources, the Third Institute of Oceanography, State Oceanic Administration, Xiamen, 361005, Fujian, China.

[2] Department of Environmental Health, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou, 510515, Guangdong, China.

[3] Key Laboratory of Global Change and Marine-Atmospheric Chemistry, the Third Institute of Oceanography, State Oceanic Administration, Xiamen, 361005, Fujian, China.

[4] Life Science College, Xiamen University, Xiamen, 361005, Fujian, China.

[#] **Corresponding Author:** Zongze Shao, Mail address: No.184, 14 Daxue Road, Xiamen 361005, Fujian, China. Phone: 86-592-2195321; E-mail: shaozz@163.com.

¶: Authors contributed equally to this work.

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

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

129 **2.2 Chemicals and media**

130 Naphthalene (>99.8%) was purchased from Sinopharm Chemical Reagent (Shanghai, China),
131 and phenanthrene (>97%) and pyrene (>98%) were purchased from Sigma-Aldrich (St. Louis,
132 USA). The Sixteen EPA priority pollutant PAH standards, six types of surrogate standards
133 (1,4-dichlorobenzene-d4, naphthane-d8, acenaphthene-d10, chrysene-d12, phenanthrene-d10,
134 and perylene-d12) and an internal standard (m-terphenyl) were purchased from AccuStandard
135 (New Haven, USA). Surrogate and internal standards were used for quantifying procedural
136 recovery and target PAH quantification, respectively. Crude oil was obtained from Iraq and
137 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 rate quantification by GC-MS

To determine the PAH-removal rates 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 rate was calculated according to the formula:

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

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.

2.7 PCR-DGGE

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

2.8 Bacterial isolation, identification and phylogenetic analysis

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

2.9 Nucleotide sequence accession numbers

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

3 Results

3.1 PAHs in sediments

GC-MS quantification indicated that the total concentration of the sixteen targeted PAHs (Σ_{16}PAH) ranged from 2.02 to 41.63 ng g^{-1} dw (Table S2) and was decreased in the sediments 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 rates 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 rates of phenanthrene and pyrene at 25 ℃ were higher than those at 15 ℃ (Fig. 4). Notably, the consortia of the northernmost site (BN12) generally displayed high rates at 15 ℃ and 25 ℃. In contrast, the removal rates 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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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 rates 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 ℃.

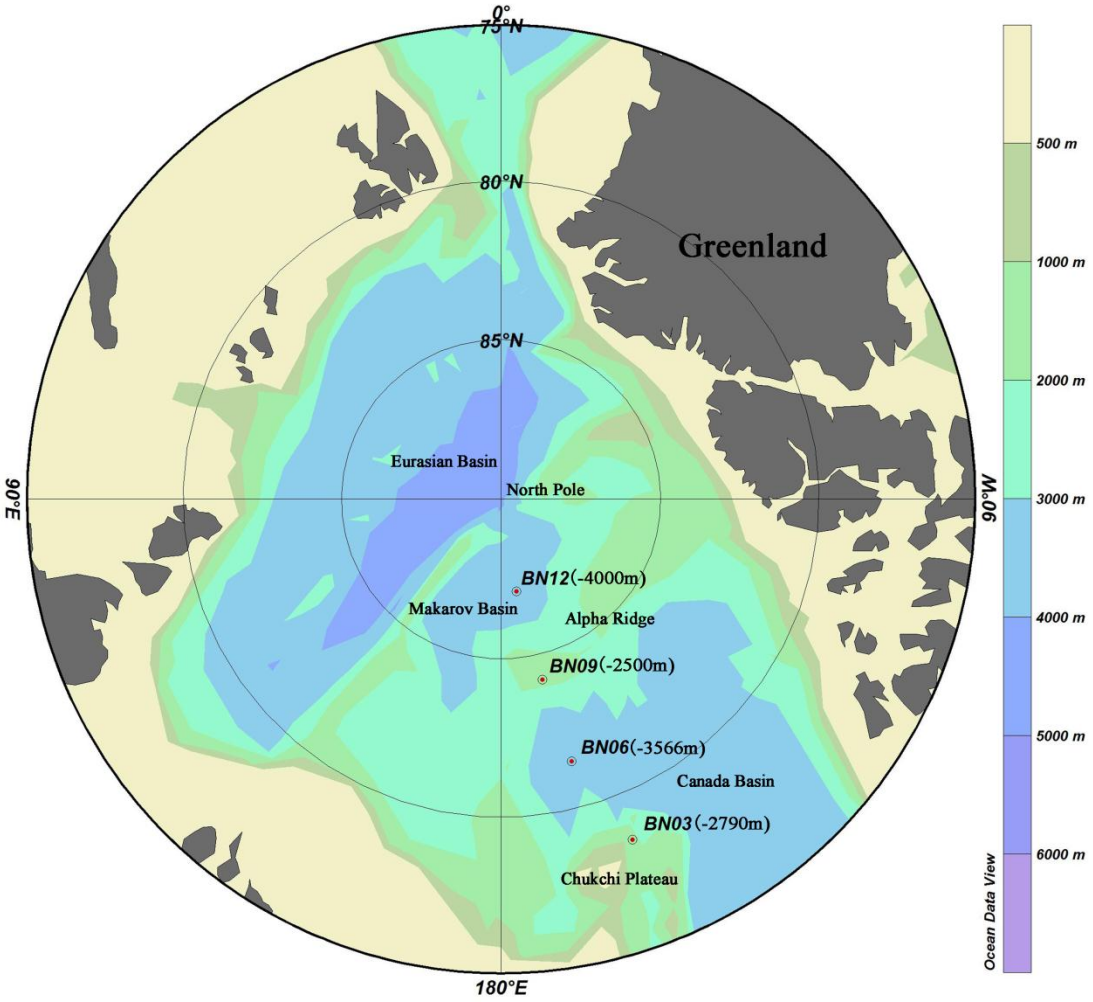
765

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.

769

770

771 Fig.1



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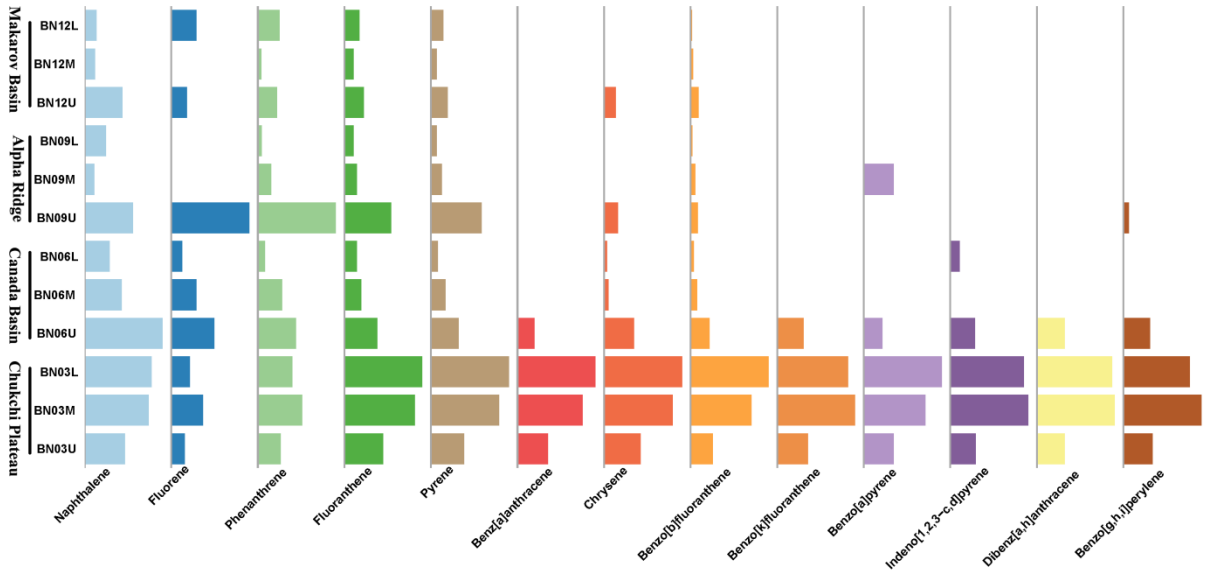
774 Fig. 2



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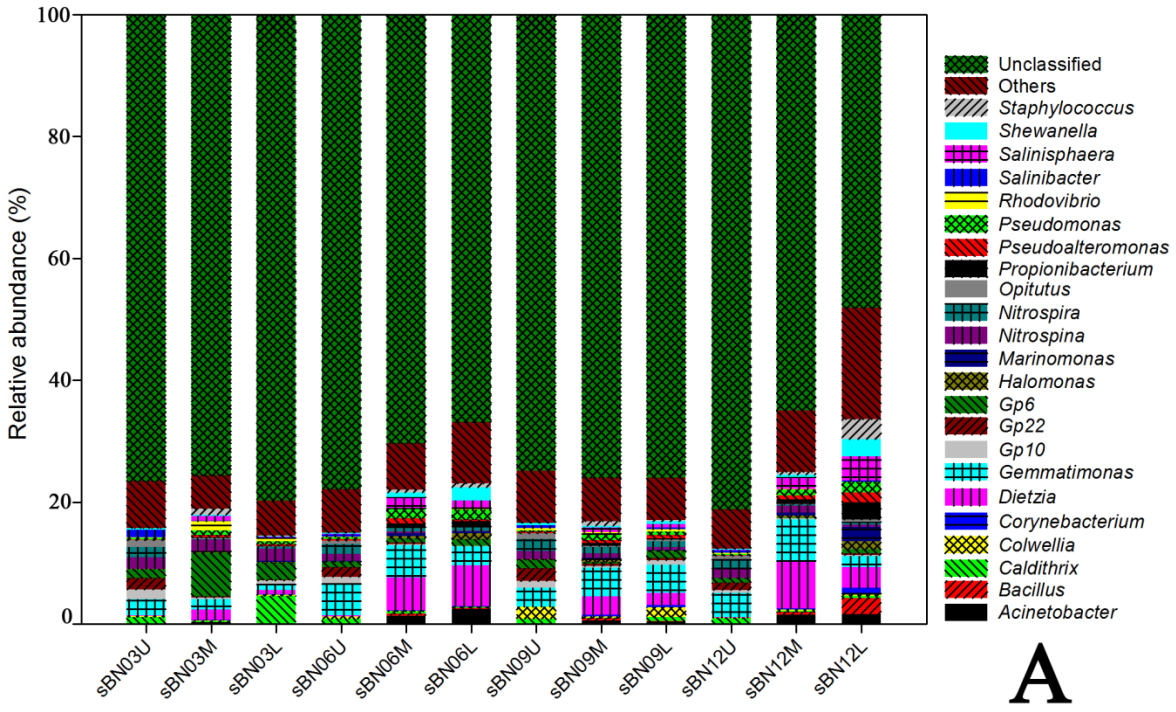
777 Fig.3



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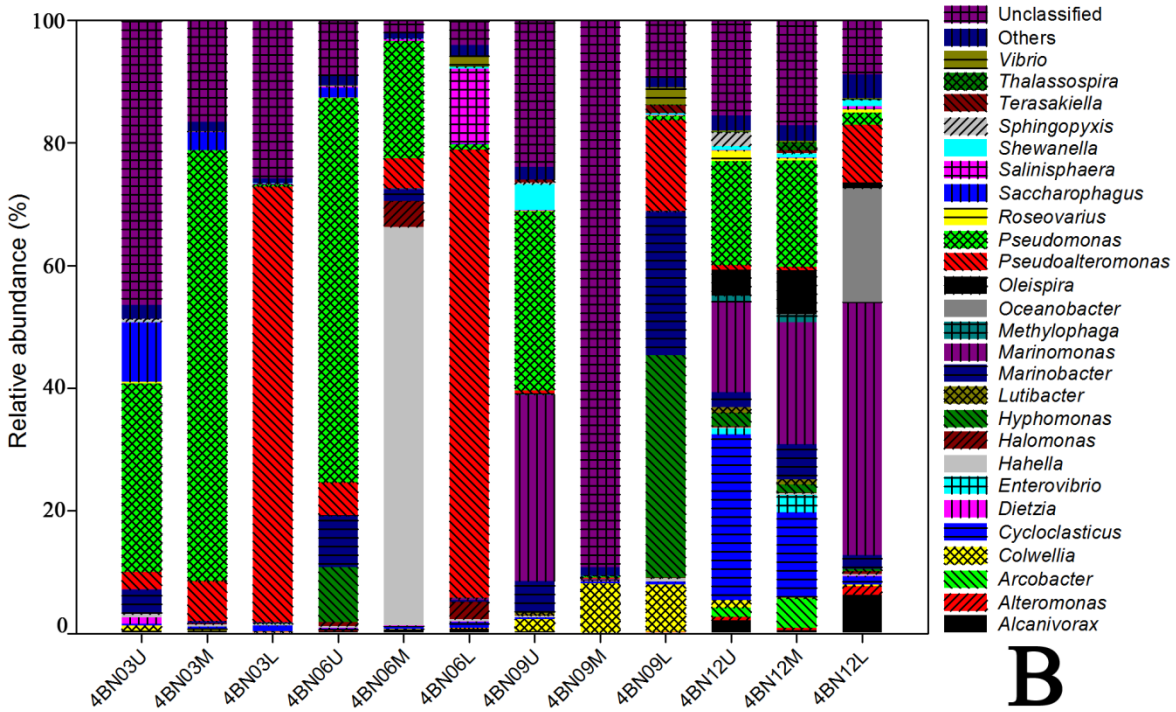

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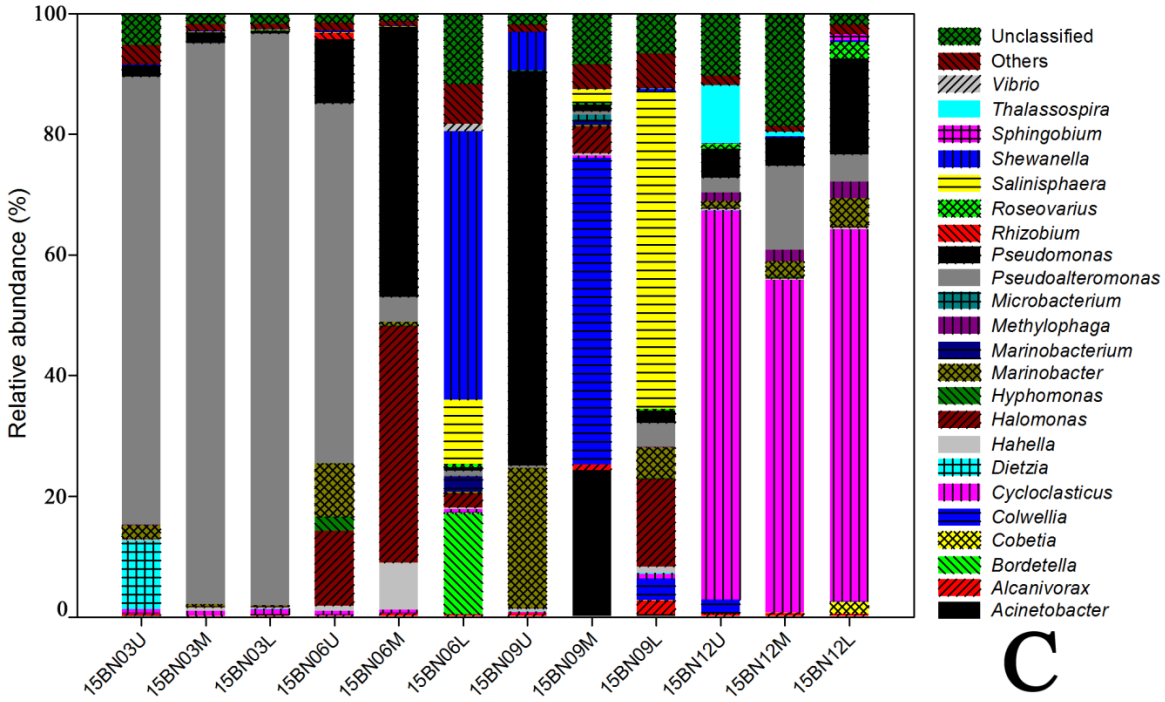
780 Fig.3 (continue)



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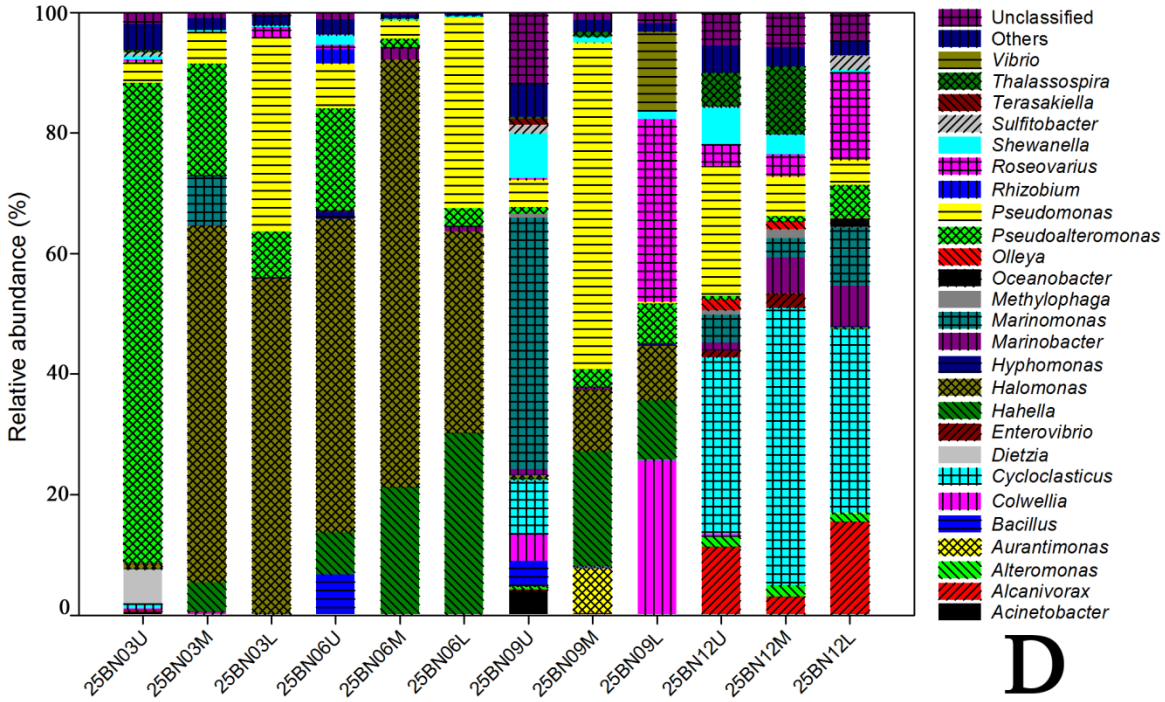

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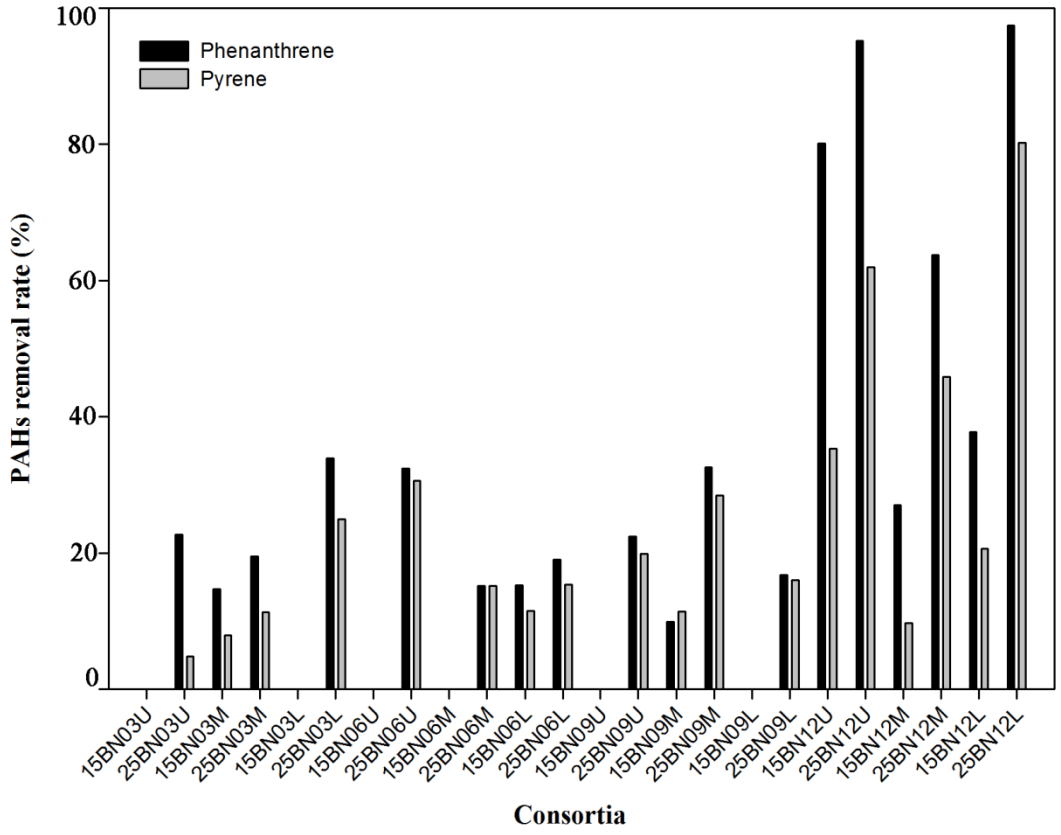
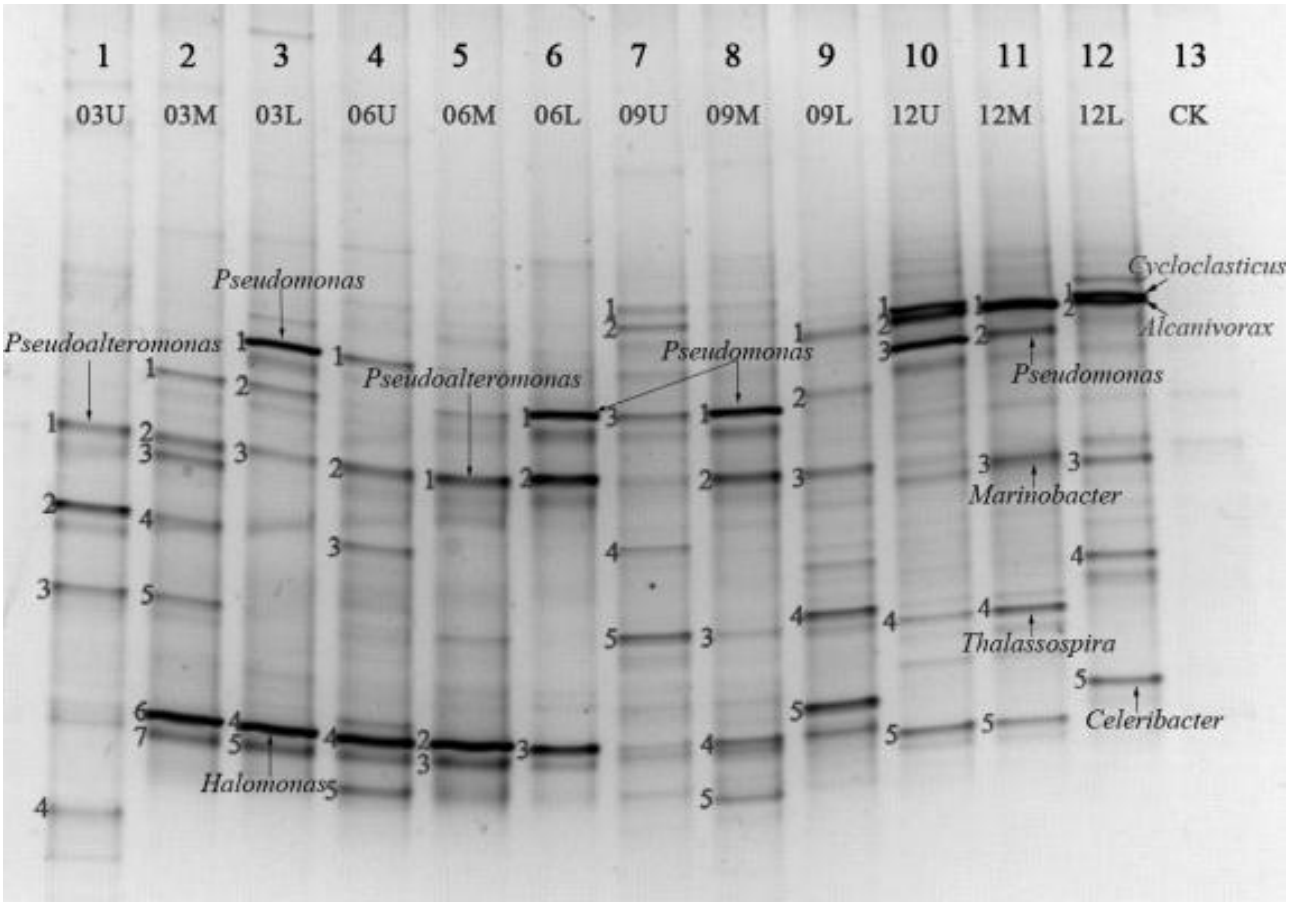
783 Fig. 4



784

785

786 Fig. 5



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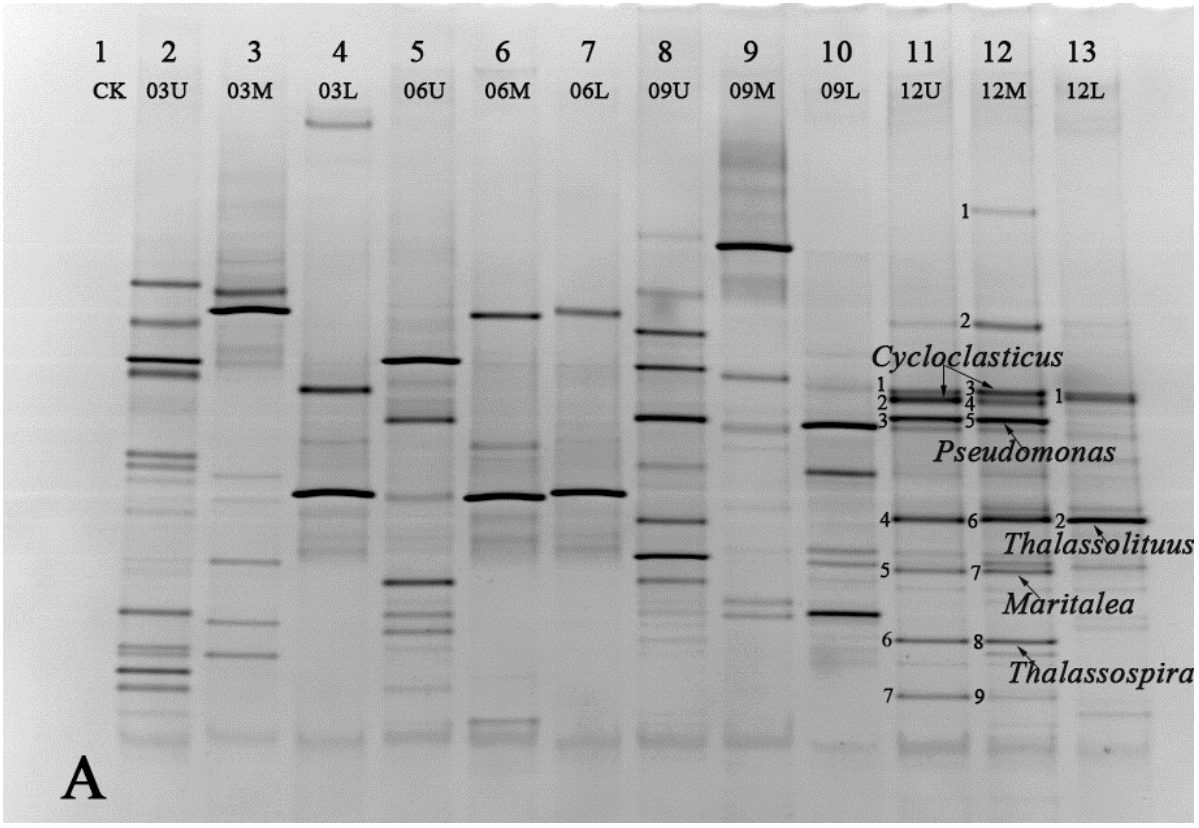
788

789 Fig. 6



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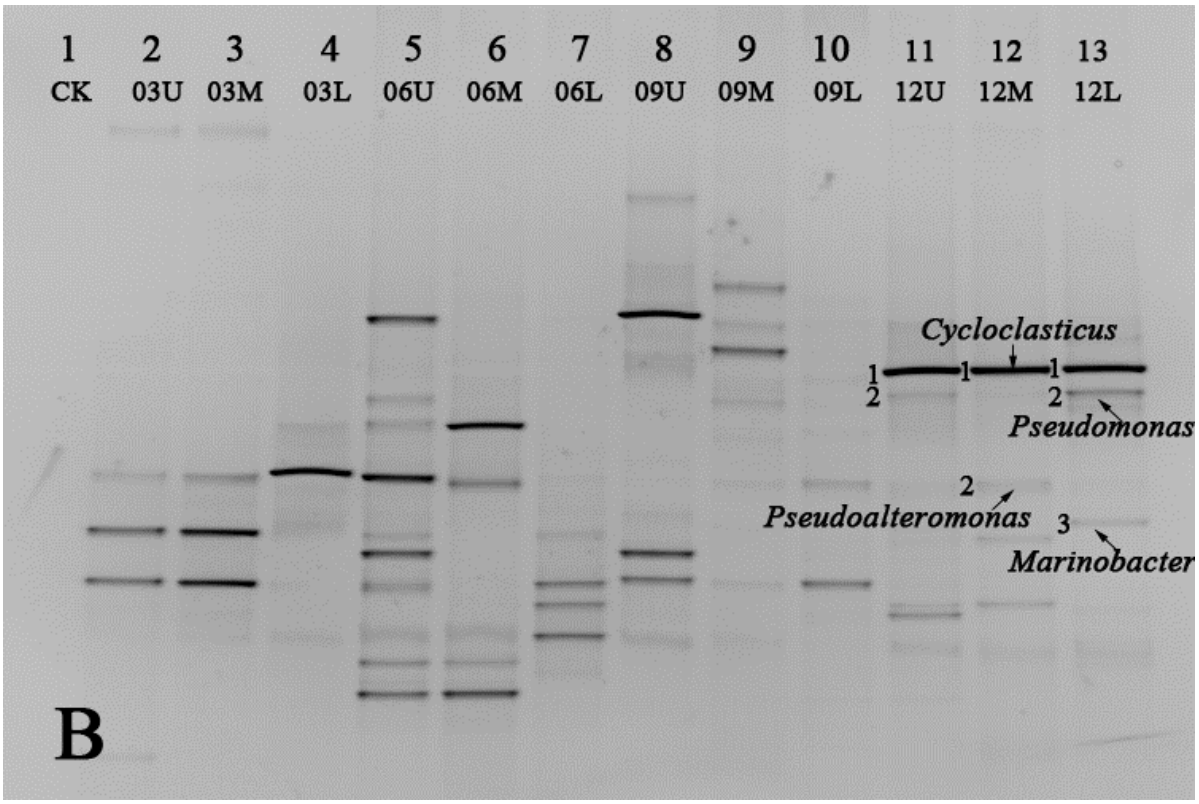

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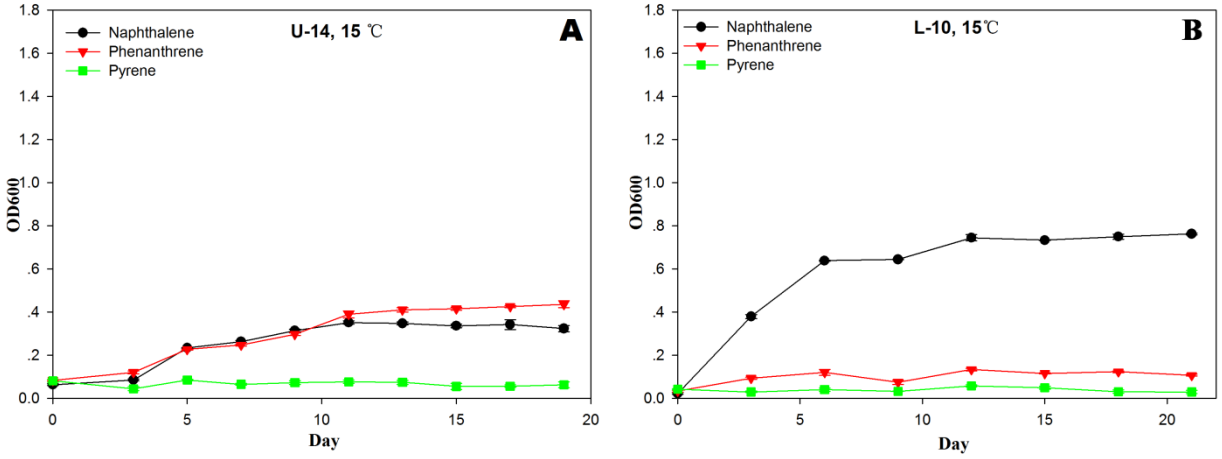
Fig. 7


A
U-14, 15 ℃
Naphthalene
Phenanthrene
Pyrene
OD600
Day
B
L-10, 15℃
Naphthalene
Phenanthrene
Pyrene
OD600
Day