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Interactive comment on "Distribution of PAHs and the PAH-degrading bacteria in the deep-sea sediments of the high-latitude Arctic Ocean" *by* C. Dong et al.

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Dear 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 American Journal Experts 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 second paragraph in page 13988 as follow “Therefore, these allochthonous PAHs could be served as carbon sources for various PAH-degrading bacteria in Arctic Ocean 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)

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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 “which 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, four sites representing these four typical geographical regions were chosen, including sites BN03, BN06, BN09 and BN12 (Fig. 1, Supplement 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

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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 -1oC to 4oC (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.

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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 Inc. Surrogate and internal standards were used for quantifying procedural recovery and target PAHs quantification".

After confirming with the co-author Dr. Liping Jiao, target PAHs quantification details were described below.

"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 1 mg/L 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 clean-up extract was further concentrated to 1 ml under a gentle N2 stream. Finally, 0.05 mL of 1 mg/L m-terphenyl was added into the extract as internal standard just before analysis.

All the samples were analyzed using GC-MS run in 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

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molecular ion. Five calibration standard solutions (0.01-500 mg/L) containing PAHs standard, internal standard and surrogate compounds were carefully prepared and calibration curve was made. The mean of the relative response factors (RRFs) for each target PAH and surrogate compounds were 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 of each type of target PAHs is from 0.13 to 0.97 ng g-1 dw."

We will supplement these 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, Supplement Table S5), PAH-degrading bacteria of Pseudomonas, Pseudoalteromonas, Cycloclasticus, and Halomonas always were dominated at 4oC, 15oC and 25oC enrichments no matter what mixture carbon source or sole PAHs was used.

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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 4oC enriched cultures were transferred into fresh media and enriched twice at 15°C and 25°C, 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

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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:

Removal rate (%)={[tPAH-(rPAH/R)]/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 15oC 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°C enriched cultures, particularly in the cultures from BN12 site. Thus, we chose 15°C enrichment cultures as the representatives to isolate Cycloclasticus.

We could not exclude that PAHs were originated from natural oil seeps, especially in

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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 "The Cycloclasticus bacteria were found in all the twelve 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 Supplement Table S5 has presented the most abundant bacteria in the 4oC, 15oC and 25oC consortia, we appended "(see Supplement Table S5)" behind ". . .. . .were also found as dominant members in some PAH-degrading consortia in this report" (lines 22-23, page 14002).

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

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PAHs initial hydroxylation and intermediate metabolism steps (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 15oC and 25oC.

A.: Inserted. Thanks.

The reason of choosing 15°C is to select the psychrotolerant PAH-degrading bacteria which grow very slowly under 4°C, but usually have optimal growth temperatures above 15°C. Thus, we shifted their enrichments to 15°C instead of 4°C. The enrichment under 25°C 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°C (Fig 5 and Supplement Table S5).

Interactive comment on Biogeosciences Discuss., 11, 13985, 2014.