Reply to Reviewer 1 comments

SPECIFIC COMMENTS
Abstract: -
The top-down control is not demonstrated in this work, you cannot mention this so securely as a major driver, neither as a result of your work. Same remark for the discussion section.

Answer. Yes indeed, so we replaced this sentence by "*We found that "bottom-up" control accounted for a large part in the variability of phytoplankton productivity*". We also modified the discussion in this way and only suggested the top-down as a potential factor.

Introduction:
 - The part related to climate change is really oversized when compared to real scientific questions addressed by this paper in its current version. Moreover, the consideration of PAH rather balance the study towards local changes because of human activities. This introduction should at least be less oriented towards climate change and present the challenge of understanding the functioning of such continental to marine environments interface in a context of growing anthropization, which seems to fit better to the design and results of the study.

Answer. We deleted the whole paragraph referenced to global change and added the challenge put forward by this remark.

- The consideration of PAH is not mentioned in the introduction. It is surprising to see it appear in the methods section without any mention in the objectives! The results and the discussion related to PAH are interesting, but you need to include it in your objectives, otherwise it is really hard to see how useful it can be to consider PAH regarding to your scientific objectives. This comment and the previous one are really connected, they should be taken into consideration together. The way PAH are mentioned in the abstract, the importance of oil-related compounds is obvious, this should be presented in this way in the introduction.

Answer. We agree with the Reviewer. We thus added two paragraphs dedicated to PAHs in the introduction:
"*Local anthropogenic inputs of organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) may also affect bacterial diversity and activities (Lekunberri et al., 2010; Rodríguez-Blanco et al., 2010; Jiménez et al., 2011). Indeed, PAHs, which can comprise as much as 25–35 % of total hydrocarbon content in crude oils (Head et al., 2006), are among the most abundant and ubiquitous pollutants in the coastal environment (González-Gaya et al., 2016). These compounds are recognized by the European and US environmental agencies as priority pollutants for the aquatic medium due to their toxicity, persistence and ability to accumulate in the biota (Kennish, 1992). Hence, the presence of PAHs in the marine environment may induce an increase in the indigenous populations of marine bacteria that can break down and utilize these chemicals as carbon source, the so called "PAH-degrading bacteria" or "PAH degraders". These bacteria are generally strongly selected in oil-impacted ecosystems, where*

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they may account for 70 to 90% of the total bacterial community (Head et al., 2006; Gutierrez et al., 2014)."

"Also, Terminos Lagoon is potentially impacted by PAHs, which may come from a diversity of sources including sea-based activities (spills from ships, platforms and pipelines, ballast water discharge, drilling...) but also rivers, surface runoffs and the atmosphere that carry various urban and industrial wastes (fuel combustion, traffic exhaust emissions...). Nevertheless, to our knowledge, little is known about the PAH content in this ecosystem. Even though Noreña-Barroso et al. (1999) have reported PAH concentrations in the American Oysters Crassostrea virginica and Rendon-Von Osten et al. (2007), PAH concentrations in surface sediments, no data are currently available about dissolved PAH concentrations in surface waters of the Terminos Lagoon."

In the objectives, we also added "*dissolved PAHs*" and "*including PAH-degrading bacteria*".

We removed one reference from the reference list:

Aguayo, P., Gonzalez, C., Barra, R., Becerra, J., and Martinez, M.: Herbicides induce change in metabolic and genetic diversity of bacterial community from a cold oligotrophic lake, World journal of microbiology & biotechnology, 30, 1101-1110, 2014.

And we added several references dealing with PAHs/PAH-degrading bacteria:

González-Gaya, B., Fernández-Pinos, M. C., Morales, L., Abad, E., Piña, B., Méjanelle, L., Duarte, C. M., Jiménez, B., and Dachs, J.: High atmosphere-ocean Exchange of semivolatile aromatic hydrocarbons, Nature Geoscience, 9, 438-442, 2016.

Gutierrez, T., Rhodes, G., Mishamandani, S., Berry, D., Whitman, W. B., Nichols, P. D., Semple, K. T., and Aitken, M. D.: Polycyclic Aromatic Hydrocarbon Degradation of Phytoplankton-Associated Arenibacter spp. and Description of Arenibacter algicola sp. nov., an Aromatic Hydrocarbon-Degrading Bacterium, Applied and Environmental Microbiology, 80, 618-628, 2014.

Head, I. M, Martin Jones, D., and Roling, W. F. M.: Marine microorganisms make a meal of oil, Nature, 4, 173-182, 2006.

Jiménez, N., Viñas, M., Guiu-Aragonés, C., Bayona, J. M., Albaigés, J., and Solanas, A. M.: Polyphasic approach for assessing changes in an autochthonous marine bacterial community in the presence of Prestige fuel oil and its biodegradation potential, Applied Microbiolal Biotechnology, 91, 823-834, 2011.

Lekunberri, I., Calvo-Díaz, A., Terira, E., Morán, X. A. G., Peters, F., Nieto-Cid, M., Espinoza-González, O., Teixeira, I. G., and Gasol, J.M.: Changes in bacterial activity and community composition caused by exposure to a simulated oil spill in microcosm and mesocosm experiments, Aquatic Microbial Ecology, 59, 169-183, 2010.

Noreña-Barroso, E., Gold-Bouchot, G., Zapata-Perez, O., and Sericano, J. L.: Polynuclear Aromatic Hydrocarbons in American Oysters Crassostrea virginica from the Termines Lagoon, Campeche, Mexico, Marine Pollution Bulletin, 38, 637-645, 1999.

Rendon-von Osten, J., Memije, M., Ortiz, A., and Benitez, J.: Potential sources of PAHs in sediments from Terminos lagoon, Campeche, Mexico, Toxicology Letters, 172, S162, 2007.

Material and methods:
- Section 2.6 and later all along the manuscript: flow cytometry cannot distinguish bacteria from archaea, thus most of the "bacterial" should be replaced by "prokaryotic" or "heterotrophic prokaryotes".

Answer. We have done the replacement in the whole text when appropriated.

- Section 2.9: Could you explicit the calculation of this MPN? The way such calculation is performed after two weeks of incubation sounds weird, how is the number of bacteria enumerated after two weeks related to the initial sample? Also refer to my corresponding comment in the result section.

Answer. Sorry, but the reviewer's remark is not very clear. The principle of the MPN technique is based on serial dilution on the reference sample to be analysed. Therefore, the calculation takes into account the initial number of bacteria and the dilution where resazurin changed color. It makes no sense to count the bacteria after two weeks incubation and use this count in the calculation. Here, the MPN is giving an estimation of the most probable number of bacteria able to degrade the mix of six PAHs as sole carbon source within two weeks. We reassure the reviewer that the use of classical MPN table based on symmetrical dilution series with a constant, table-specified dilution factor, as explained in the material and method section.

A reference has been added in the material and method section, in case the reader wants to have more information on this method classically used in microbiology.
Alexander, M., 1982. Most probable number method for microbial populations. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2, 2nd ed. American Society of Agronomy, Madison, WI, pp. 815-820.

Results: - Lines 279-280: very consistent, but could you indicate the approximate value of the ratio in this area?

Answer. We indicated in the text the Phaeo:CHL ratio of the area (<25%)

- Lines 292-294: the correlations concerning aminopeptidase activities are very weak, it should not be presented at the same level than the ones concerning phosphatase activities, which are stronger. This probably means that there is indeed a correlation between these variables, but they are not linear and Spearman's correlation tests the linearity of the relation. You should try non parametric and non linear correlation analyses to further precise the link between these variables.

Answer. We now precised ".... to a lesser extent, between phosphatase activities...."

- Lines 296-299: Need to explain how such percentage is obtained. As it is explained in ,the current version of the manuscript, one could understand that you counted bacteria in your 2 week enrichments and divided this number by the number of bacteria in your initial samples... This does not sound correct.

Answer. See our previous response on - Section 2.9 for this remark.

- Lines 300-303: I don't understand the cause-to-consequence relationship you mention here (second part of the sentence). You should split the sentence into two very descriptive ones.

Moreover, the PAH distribution should be presented at the beginning of this paragraph, then followed by PNM counts, and finally the correlation analysis. Thus the two components of the title should also be switched.

Answer. We agree with the reviewer. We modified this paragraph 3.5 accordingly:
We replaced the paragraph title "*3.5 Estimated abundance of bacterial PAH-degraders and PAH concentrations*" by "*3.5 Dissolved PAH concentrations and estimated abundance of bacterial PAH-degraders*"

And we replaced the whole concerned paragraph:
"*Quantification by MPN counts showed high enrichment of PAH-degraders close to Palizada river (estimated at 4.6 104 cells mL^{-1}, equivalent to 4.4 % of the total bacterial abundance) (Fig. 5A). Lower values were found close to the Chumpan embouchure (estimated at 4.7 103 cells mL^{-1}, equivalent to 0.2 % of the total bacterial abundance), and commonly represented less than 0.1 % of the bacterial abundance in the rest of the lagoon. Quantification by MPN counts showed significant even if low spearman rank correlation with dissolved total PAH concentrations (R=0.37, p<0.05, n=35), mainly because of PAH distribution (Fig. 5B) showing higher concentrations close to the El Carmen inlet (332 ng L^{-1}) and relatively lower concentrations close to Palizada river (187 ng L^{-1}) and to the Chumpan embouchure (166 ng L^{-1}). Correlations (p<0.05, n=35) were stronger with PP (R=0.65) and CHL (R=0.53). PAH concentrations were generally lower in the rest of the lagoon (<130 ng L^{-1}).*

By the paragraph:
"*Dissolved total PAH concentrations (Fig. 5A) were higher close to the El Carmen inlet (332 ng L^{-1}) and relatively lower close to Palizada river (187 ng L^{-1}) and to the Chumpan embouchure (166 ng L^{-1}). They were generally lower in the rest of the lagoon (<130 ng L^{-1}). Quantification by MPN counts showed high enrichment of PAH-degraders close to Palizada river (estimated at 4.6 104 cells mL^{-1}, equivalent to 4.4 % of the total bacterial abundance) (Fig. 5B). Lower values were found close to the Chumpan mouth (estimated at 4.7 103 cells mL^{-1}, equivalent to 0.2 % of the total bacterial abundance), and commonly represented less than 0.1 % of the bacterial abundance in the rest of the lagoon. Quantification by MPN counts showed significant even if low spearman rank correlation with dissolved total PAH concentrations (R=0.37, p<0.05, n=35). Correlations (p<0.05, n=35) were stronger with PP (R=0.65) and CHL (R=0.53).*"

Consequently, we inverted the order of Fig. 5A and 5B:
Figure 5: as Figure 2 for A. total dissolved PAHs (ng L-1) and B. the most-probable-number (MPN in count)

Discussion:
- Lines 346-348: The corresponding figures are puzzling because, yes, the normalized productivity per cell seems to be higher, but since the CHL concentration is also lower, one could think that the productivity of the area expressed by unit of volume could be lower. Since you have CHL concentration per liter and since the productivity is expressed is expressed by unit of CHL, I suggest that you calculate a productivity by volume of water in order to better compare the productivity of the two sites. According to your graph, you have

less than a 2-fold difference in CHL concentration between the two sites but a 6- to 7-fold difference in C fixation rates. Thanks to such analysis, your conclusion will be more robust.

Answer. Thanks for this remark. In fact, we have actually about 2-fold difference in CHL concentration between the two sites but about the same ratio and not 6- to 7-fold difference in C fixation rates. So, for similar amount of nutrients (and DOM and DOP also…) we have similar production for half chlorophyll… Here is the table for mean values of the 2 groups (I included 4 stations in each group…)
I also added a sentence in the text concerning this comparison.

	CHL	P^b_m	PP max	NO3	PO4	NH4
	$mg.m^{-3}$	$mgC.mgCHL^{-1}.h^{-1}$	$mgC.m^{-3}.h^{-1}$	µM	µM	µM
mean G palizada	6.34	4.34	27.5	0.02	0.08	0.05
mean G inlet	3.95	7.65	30.2	0.03	0.08	0.06

- Lines 352-353: Could you provide references to support such hypothesis since you did not measure it?

Answer. We now modified this paragraph to modulate the affirmation.

- Lines 359-361: This sentence should be rewritten in a more prudent way since it is only speculation, you have no clue for the top down control and even though you have PAHs concentrations, the direct link with phytoplankton productivity remains to be demonstrated.

Answer. We now modified the sentence as follow *"Finally, it is clear that bottom-up (nutrients and humic substances) drove the differential responses of phytoplankton productivity in the eastern and western part of the lagoon, certainly in conjunction with grazing activity (top-down control")*.

- Lines 378-380: sounds weird to justify a recent study by an old one... The old reference could be removed without making the sentence meaningless or doubtful.

Answer. We deleted the reference.

- Lines 383-386: These Redfield ratios were not presented in the results sections. They are meaningful and should be presented extensively!

Answer. We added the map for the distribution of this ratio in the lagoon.

- Lines 410-413: That could appear contradictive. If you suppose that higher concentrations of DOC but lower aminopeptidase activity suggest a higher amount of labile organic matter for bacteria, you should clearly state it.

Answer. We now explained that higher DOC concentrations associated with lower aminopeptidase activity suggest a higher amount of labile organic matter for bacteria. The high aminopeptidase activity in the Palizada River plume confirmed the presence of recalcitrant organic matter from terrestrial origin, as opposed to minimum activities in Puerto Real marine waters or in Candelaria mouths, where DOC concentrations were maximal.

- Lines 425-427: So vincinity of Palizada river = phosphatase activity but P-depleted zone, meaning very low P-availability for phytoplanktonic growth, which seems consistent with le smaller C fixation rates observed in this zone, am I right? If yes, such a link between nutrients and biological productivity could be added to this discussion, this would greatly strengthen the end of the first sub-section which was up to now very speculative. Moreover, this would also strengthen the links between the geochemical and the biological sides of this paper which appear very few connected for the moment.

Answer. Thanks for this pertinent remark that we included in the text.

- End of section 4.3: A small discussion could be added about distance-based effects and community turnover: according to your data, one could think that strong local selective pressures led to strong shift in the community composition, instead of having progressive gradients and/or fast dispersion that would rather just promote a turnover within the active fraction. Such thinking points out the existence of strong driving pressures that you did not measured in your study, since you do not have such strong correlation

Answer. As describe above, we changed the sentence into: "These results indicated that most of the free-living bacterial community detected by molecular fingerprinting (DNA-based) were active (RNA-based) among the lagoon, with the exception of the local transition zones between the lagoon waters and the coastal (El Carmen inlet) or rivers (Palizada and Candelaria)."
This sentence points out that some transitions zones strongly affect bacterial diversity and activities. We agree with the reviewer's comment but it is difficult to state on either a strong local selective pressure or progressive gradient driving the strong shift of the community observed in this study. Because our dataset aimed to give an overview of the entire lagoon rather than focusing on the several transition zones of the lagoon, we decided to be cautious and not over interpreting our results.

- Lines 460-466: These two sentences should rather be placed at the beginning of the previous paragraph, before discussing about community structure.

Answer. We moved these sentences at the beginning of the paragraph 4.3 as recommended

- Lines 475-477: Adding a short discussion about "distance-based" similarities in present and active communities, as proposed in a previous comment would perfectly fit with this discussion.

Answer. We added the short discussion suggested

- The link between the severe drought and the potential predictive aspect of this study is not mentioned all along the discussion section, whereas it represents most of your introduction. You need either to remove this "climate-change" part in the intro, or discuss it in the discussion.

Answer. We modified the introduction and discussion in consequence

Conclusion
- Lines 512-514: You cannot say that as it was unambiguous, you did not even measure any top-down parameter.

Answer. We modulated our affirmation, in indicating that we supposed a possible shift. In fact we have some signals that confirm this hypothesis; it is the reason why we chose to keep it in the text.

- Lines 514-517: This sentence does not sound correct since you measured C fixation, thus phytoplankton activity. To measure growth, you need repeated measures in time, that you do not have. So you can definitely not state that there is no growth. I understand it is probably not what you wanted to say, but your sentence could be misinterpreted

Answer. We replaced growth by C-fixation

- Lines 528-530: again, you are over confident in your hypothesis: "seems to support", you do not have any temporal data to support this hypothesis.

Answer. done

TECHNICAL CORRECTIONS

Answer. All the 15 remarks have been taken into account and fixed